

On the Development of *Cucumaria echinata* v. *Marenzelleri*.

By

Hiroshi Ohshima.

With Plates 8 and 9 and 11 Text-figures.

CONTENTS.

	PAGE
1. INTRODUCTORY REMARKS	173
2. PREVIOUS STUDIES ON ALLIED FORMS	177
3. METHODS OF INVESTIGATION	179
4. BREEDING SEASON	181
5. GENITAL ORGANS	182
6. SPAWNING	186
7. NEWLY-SHED EGGS	188
8. CLEAVAGE	190
9. BLASTULA	192
10. GASTRULA	195
11. DIPLEURULA	199
12. DOLIOLARIA	204
13. METADOLIOLARIA	218
14. PENTACTULA	221
15. YOUNG	224
16. SUMMARY	237
17. BIBLIOGRAPHY	240

1. INTRODUCTORY REMARKS.

THE following passages, which are to be found in the diary of the Marine Biological Station of Misaki, concern the spawning of *Cucumaria echinata*, and are written chiefly by the late Professor Dr. Kakichi Mitsukuri.

' June 18, 1899. Mitsukuri arrived at the Station to-day, being informed of the fact that in these days Messrs. Aoki and Tsuchida had observed the spawning of *Cucumaria echinata*.'

' June 20. If some freshly caught specimens of *C. echinata* are kept in a glass vessel, it is almost certain that they will spawn in the evening.'

' July 21. *C. echinata* spawned!'

' July 29. *C. echinata* caught in the morning began to spawn at 5 p.m.'

' August 11, 1902. Kuma Aoki dredged several scores of *C. echinata* at the mouth of the inlet of Koajiro. They began to spawn at 7.30 p.m. and went on till about 10.30 p.m.; a very large number of eggs were spawned. Mitsukuri engages in the study of them.'

In his memoir on pedate holothurians (31, 1912, p. 242) the late professor has recorded with reference to the above facts as follows: ' In the breeding season (the summer) the ripe individuals throw out reproductive elements. The males shoot forth the spermatic fluid, after which the females begin to shed eggs, which easily undergo development under observation.'

So far as the results of his study are concerned, unfortunately no report was made, except his two short addresses delivered at the monthly meetings of the Tōkyō Zoological Society. The contents of those addresses can now be recovered only from unpublished notes made by a member who attended the meetings.

The first address was given on December 16, 1899, under the title ' General Account of the Embryology of Holothurians '. The notes may be translated as follows :

' To obtain eggs of *C. echinata* it is necessary to tease the animal and not to change the water. Animals captured in the morning will lay eggs in the evening. They are in an extended posture while laying eggs; the genital products are emitted from the interradially situated genital pore in the form of a streak. The amount of the products of each emission is very remarkable. No peculiar points are noticeable in the segmentation of the eggs. In the five-tentacled stage a pre-oral

hood is formed containing a mass of food-yolk of a green colour. The first pair of pedicels appear on the ventral side, and at this stage large calcareous plates, which differ in shape from those found in the adult, appear in the interradii. Next to the five primary tentacles (Text-fig. 1, I) three more (II_1 , II_2 , II_3) arise, of which in most cases the right-hand one (II_1) appears first. The larva attains about 2 mm. in length without having developed the two remaining tentacles (III). The manner of the branching of the tentacles seems to follow a certain rule, just as in the

TEXT-FIG. 1.

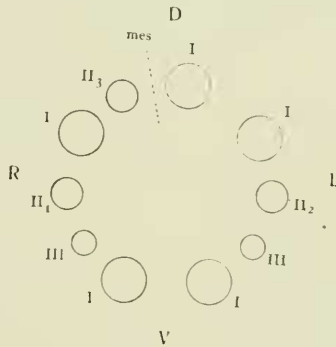


Diagram showing the order of appearance of the tentacles. Anterior view. Larger circles (I) represent the primary tentacles; medium-sized (II), secondary ones appearing next; and smallest ones (III), those last to appear. *mes.* = position of the dorsal mesentery. (After Mitsukuri.)

phyllotaxis among plants, and the second pinnule is the largest of all (Text-fig. 2).

'The first pair of pedicels are followed by the third (Text-fig. 3, 3) appearing on the left side of the midventral radius, then the fourth (4) appears on the ventral side of the right ventral radius. A further increase of pedicels may be seen in the diagram. To some extent the pedicels increase forwards from the height of the first pair, while later some appear behind it.'

The second report was contained in his address on the change

of calcareous deposits in *Holothuria vagabunda*, read on February 21, 1903. Here he spoke again on the sequence of the appearance of the secondary tentacles. Judging from the figure he indicated a difference from his former statement, since the dorsal tentacle (Text-fig. 1, II₃) was shown as the first to appear among the secondaries.

Having been engaged in the study of holothurians since the lamentable death of Professor Mitsukuri on September 17, 1909, I have made a new and careful examination of the subject. On August 12, 1910, my first attempt ended in failure because the spawning season was already over in that year. During

TEXT-FIGS. 2 & 3.

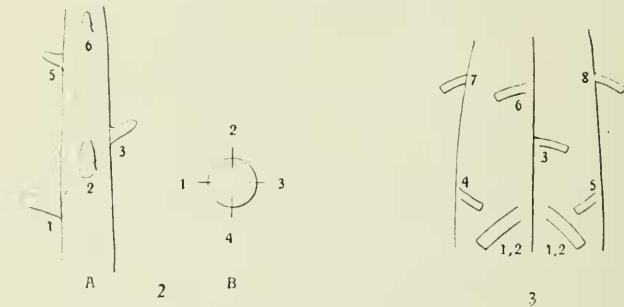


Diagram showing the manner of branching in tentacles of the young. A=Side view of a part of the stem to show the spiral arrangement of 'pinnules' 1, 2, 3. B=Profile of the stem to show positions of pinnules. (After Mitsukuri.)

Diagram showing the order of appearance of pedicels in the young. Ventral view. Numbers 1-8 indicate the order of appearance. (After Mitsukuri.)

the next few years I was unable to visit Misaki at the right season, but at last, on July 25 and August 1, 1916, I was fortunate enough to observe the animal spawn and to rear the larvae. By the kindness of Professor Dr. I. Ijima I was permitted to examine the valuable material left by the late professor, on which, and on my own collection, my studies have been based; and incomplete though it may be, owing to several gaps in the developmental stages in the materials available for examination,

I believe I have been able to throw some light upon the embryology of the group.

The present work was started while in the Zoological Institute of the Tōkyō Imperial University, but the greater part of it was carried on in the biological laboratory of the Fifth High School in Kumamoto, and it was completed in November of 1918. Owing, however, to the pressure of various affairs it could not have been published before I had left Kumamoto the next summer. The manuscript was then brought with me to London, and has been subjected to Professor E. W. MacBride's kind and careful revision. It is my pleasant duty here to express my hearty thanks to Professor I. Ijima and Professor S. Goto for their kindly supervision, and to various others for their help. Further, I extend my gratitude to Mr. K. Yoshioka, Director of the Fifth High School, by whose favour I have been able to enjoy many facilities to assist me in my studies. Lastly, to Professor MacBride, to whose courtesy the appearance of this paper is entirely due, I beg to tender my deepest indebtedness.

2. PREVIOUS STUDIES ON ALLIED FORMS.

So far as the embryology of apodous holothurians and our knowledge of typical auriculariae are concerned, the famous works of J. Müller, Metschnikoff, Semon, Bury, Mortensen, and Clark are unrivalled; but our knowledge of the pedate forms is meagre and, for the most part, fragmentary. This meagreness is due, I think, partly to the fact that artificial fertilization is very difficult,¹ and partly to the shortness of the larval stage, the auricularia stage being usually omitted,² so that the larva easily escapes the eyes of investigators of the

¹ Clark (7, 1898, p. 58), Mitsukuri (30, 1903, p. 11), and Edwards (12, 1909, p. 212) never succeeded, while Selenka's experiment (45, 1876, p. 157) resulted in the malformation. Only one case of successful artificial fertilization is recorded by Mortensen in *Holothuria nigra*, though in this case only a small percentage of the eggs developed (35, 1913, pp. 17-18).

² *Holothuria tubulosa* and *H. nigra* pass through a typical auricularia stage (Selenka, 45; Mortensen, 35).

plankton. Among pedate holothurians, the forms which offer the materials for the study of embryology belong to two families only, i. e. Holothuriidae and Cucumariidae, both of which are chiefly dwellers in shallow water. Östergren (42, 1912, p. 338) attributed the shortness of larval life in Cucumariidae to the fact that they live near the coast. Any longer period of larval life, he thought, would expose the larvae to greater danger of being swept away by violent currents to destruction.

Again, our knowledge of their development is fragmentary, because, first, many of the observations have been made on those forms which have brooding habits in which it is not easy to secure a complete series of developmental stages, and, secondly, there are some difficulties in the technique of investigation, the egg being yolky and the larvae mostly opaque.

The embryology of pedate holothurians was first studied by Danielssen and Koren (11, 1856). Their material was identified as *Holothuria tremula*, but Ludwig (21, 1889-92, pp. 249, 251) and Mortensen (33, 1898, p. 24) alike denied this and suggested that it was dendrochirote. Later, Louis des Arts (2, 1910, pp. 9-10) and Östergren (42, 1912, p. 338) have both identified it as *Cucumaria frondosa*. Kowalewsky (17, 1867) was the second who had the good fortune to observe the spawning of *C. planci* (= *Pentacta doliolum*) and *C. kirchsbergii* (= *Psolinus brevis*); he managed to rear larvae for over ten days, which attained the pentactula with a pair of primary pedicels.

Selenka's work (45, 1876) on *Holothuria tubulosa* and *Cucumaria planci* (= *C. doliolum*) entered into a more detailed account than those of his forerunners, since he employed the paraffin-section method. Ludwig (22, 1891) most excellently explained the processes of organ formation, and elucidated many points upon the origin of various organs in *C. planci*. He escaped the failure which befell Selenka, since he was successful in obtaining well-orientated serial sections, whereas Selenka adopted the method of 'Masseneinbettung', i. e. embedding large numbers of embryos

together and trusting to chance to obtain some orientated in the right direction. But unfortunately his materials were fixed only once every day, so that there were gaps between the stages he obtained. He was able to publish only preliminary notes without figures, and no final report. In his 'Holothurien' of Bronn's 'Klassen und Ordnungen des Thierreichs' (21, 1889-92) he summarized the facts known up to that time chiefly in *Labidoplax digitata*, *Holothuria tubulosa*, and *Cucumaria plani*.

Mortensen (32, 1894) described in detail the young of a brooding form, *Cucumaria glacialis*. Another brooding form, *Phyllophorus urna*, was then studied by Ludwig (24, 1898). Lo Bianco's skill managed to keep the young of that species alive for two months outside the mother's body in a small aquarium.

In Edwards's study (12, 1909) on *Holothuria floridana* much attention was paid to the development of the ambulacral appendages, some important details in the early changes of other organs being left unnoticed. Des Arts (2, 1910) succeeded in rearing larvae of *Cucumaria frondosa* and observed some early changes and pathological accounts caused by change of temperature. The most promising work has been carried on by Newth on *C. saxicola* and *C. normani*, yet we know of his results only through a preliminary note (36, 1916).

Thus far the important works so often referred to in the present paper. Other works left unnoticed in the above enumeration will be cited later on as occasion may require.

3. METHODS OF INVESTIGATION.

The newly-shed eggs were transferred to a larger glass vessel by a pipette. The vessel was cylindrical in shape, 28 cm. in diameter and 18 cm. in height, and was filled with clean filtered sea-water. The water was changed the next morning, and was afterwards left unchanged. The vessel was tightly covered with a glass plate, and was soaked in cool well-water which was changed once or twice a day.

To kill and fix the larvae I used a chromo-acetic mixture containing a slight amount of osmic acid. Later examination showed that this fixative proved very satisfactory for the study of tissues, but for embryological purposes it is bad, since the free cells contained in the hydrocoele and enterocoele are fixed in a very expanded state, almost filling up the lumen of these vesicles. Newth used Bouin's picro-formol-acetic and Flemming's strong solution, and seems to have experienced a similar difficulty, judging from his following remarks: 'Even after the tentacles are well established, and can be protruded and retracted, their lumen is obliterated in some places by the vacuolated inner ends of their cells', and 'there is a complete suppression of the typical curved hydrocoele crescent owing to the large size and close crowding together of its lobes and to the thickness of their walls' (36, p. 636).

The late Professor Mitsukuri seems to have used acetic sublimate, and his materials proved very good for the study of those internal cavities, which remained very wide and distinct. Some larvae of *C. planici* which he obtained at Naples on March 25-7, 1898, labelled as fixed with acetic sublimate, are in a precisely similar condition to his material of *C. echinata*. Ludwig (22, p. 604) doubted the wisdom of Selenka's employment of chrom-osmic mixture, and recommended a careful alcohol method. For observations on calcareous deposits in somewhat advanced larvae the latter were simply killed in alcohol.

For the orientation of the material to be sectioned, the double embedding in celloidin-paraffin gave good results. First, the material was put in celloidin-clove-oil mixture, and then hardened with chloroform. The hardened block was then clarified with carbol-xylo and transferred into melted paraffin. Even by employing the celloidin-paraffin method of sectioning, shrinkage of the material by about at least 15 per cent. diameter is unavoidable. That is why the Text-fig. 5, which is drawn from a whole mount, is larger than other figures obtained from sections. Sections were cut of a thickness of 5μ , except in the case of the egg and quite advanced young, which were cut into sections 8μ thick.

The sections were stained with Heidenhain's haematoxylin and orange-G, and very satisfactory results were obtained even from the material which had been preserved for nearly twenty years, enabling pictures to be taken by photomicrography.

For the reconstruction of sections, the graphical method with a glass-plate with parallel lines proved very simple and quite satisfactory.

4. BREEDING SEASON.

As mentioned above the late Professor Mitsukuri observed the spawning of *Cucumaria echinata* on July 21 and 29, 1899, and on August 11, 1902, and Messrs. Aoki and Tsuchida seem to have observed it previous to June 18. I myself observed the same phenomenon on July 25 and August 1, 1916, but as stated above, on August 12, 1910, I was too late to secure the spawning individuals.

Though the animal occurs in April and May 'in such abundance that many boats dredge for them day after day, and by evening each one is loaded down with them' (Mitsukuri, 31, p. 241), no spawning takes place in the evening, and an examination of the gonads reveals the fact that they are still immature. It is strange that in July and August the animal does not occur in such abundance as in April and May. Further, from the latter part of July to the early part of August we meet with minute young provided with five to ten tentacles and pedicels of varying numbers, mingled with sand and broken shells, in which adult animals are found embedded. These young measured only 1.5-4.5 mm. in length on August 12-17, 1910, while on July 20-5, 1916, they were remarkably large and in a far advanced state of development (see Table V).

From these facts we may conclude that the breeding season begins about the middle of June and comes to an end in the early part of August, though it varies to some extent according to different years. The larger young found in the latter part of July may have developed from eggs spawned quite early in the season.

Sex-Ratio.—Selenka (45, p. 157) noticed that in

Holothuria tubulosa both sexes occur in approximately equal numbers. Lo Bianco (19, 1899, p. 476) found that in a certain locality males of *Phyllophorus urna* occur much more abundantly than females. I could pay no special attention to the sex-ratio of *C. echinata* during the breeding season. But of the specimens collected on April 9, 1914, an examination of over 3,000 individuals showed that there were 1,627 males to 1,596 females, or that the ratio of males per 1,000 females is 1,019. Both sexes thus occur in almost equal numbers.

5. GENITAL ORGANS.

In addition to the statements of systematists, such as that given by v. Marenzeller (28, 1881, p. 128), I may describe some points about the genital organs.

Genital Tubes.—Both in males and females the genital tube consists of an external epithelium, a connective tissue layer, and an internal germinal epithelium. The external epithelium, which is continuous with the peritoneum, is very thick in an inactive stage of the gonads (Pl. 8, fig. 2, *ep*), and the connective tissue layer is very thin. I have no positive evidence to prove the presence in *C. echinata* of the muscle layer which was found in *C. glacialis* (Mortensen, 32, p. 715) and *C. laevigata* (Aekermann, 1, 1901, p. 731). In *Pseudocucumis africanus*, however, I found in the specimens fixed on July 28, 1915, when the breeding season was over, rather scattered circular and longitudinal muscle fibres, a connective tissue layer, and a very high external epithelium. Though having the same habit of carrying the brood inside the body-cavity, the structure is totally different from the peculiar feature found by Clark (7, 1898, pp. 58-9) in *Synaptula hydriformis*. Here he observed a very thin external epithelium and scanty connective tissue, which, according to that observer, probably break so as to allow the ripe eggs to escape into the body-cavity.

Female Gonad.—In a still unripe condition, as was met with in the specimens collected on March 27, 1914, the female gonad is light purplish grey in colour, with a very thick wall

owing to the high external epithelium as stated above. It contains eggs of various sizes. The egg is slightly flattened and is attached to the wall of the genital tube by its broad surface (Pl. 8, fig. 2), not by a slender stalk formed of the follicular epithelium, as described by Sempér (47, 1867-8, p. 144), Jourdan (16, 1883, p. 52), and others in *Holothuria*.¹ The germinal vesicle (*n*) is large and spherical, lying eccentrically near the free end of the egg. The centre of the free surface is indicated by a minute conical process of the cytoplasm (*ma*).

The gonad in the breeding season (Pl. 8, fig. 3) has become thin-walled and yellowish in colour, containing still very small eggs as well as large ones, which latter are ready for spawning. The germinal vesicle (*n*) has now approached much nearer the free end than in the foregoing stage, leaving a thin layer of yolky cytoplasm between it and the egg-membrane. Germinal spots (*gs*) are more minute and fewer compared with those in the early stage.

The centre of the free surface is now very peculiar in structure. Here we see a compact cytoplasmic mass, of a somewhat fibrous nature, forming a short rod-like body protruding through the egg-membrane, with its free enlarged knob-like end attached to the follicular epithelium (Pl. 8, fig. 3, *ma*). Usually the proximal end reaches the germinal vesicle and becomes continuous with its membrane, but in rare cases it ends apart from the germinal vesicle, where the latter is not very near to this pole of the egg. The space between the egg-membrane and the follicular epithelium (*j*) was perhaps occupied by a gelatinous layer. This remarkable structure, which I may call a micropyle appendage,² is only conspicuous in full-grown eggs,

¹ According to Hamann (15, 1884, p. 89) *H. tubulosa* is exceptional; here a fibrous bundle of connective tissue serves to fasten the egg.

² As early as in 1851, J. Müller remarked that the ovarian egg of *Pentacta doliolum* is flat, and that at the centre of a flattened surface there is formed a yolky process which passes through the thick jelly layer investing the egg. He further observed a similar structure in some other *Holothurians*—*Thyone fusus*, *Holothuria tubulosa*, &c. ('Monatsber. Akad. Berlin', April, 1851; 'Phys. Abhandl. kön. Akad. Wiss. Berlin', 1852 (1850), p. 77, Taf. IX, fig. 8, 9; Müller's 'Arch. f. Anat. u. Physiol.', 1854, p. 60).—Jan. 28, 1921.

while in the very early stages no such structure appears (fig. 1). In a rather small one I only once made out a slight indication of this structure.

A similar feature was observed by Semper (47, p. 144). He found that in *Holothuria impatiens* and others the jelly-canal ('Mikropylkanal') of all the egg is directed without exception to the internal lumen of the ovarian tube, and in his figs. 6 and 7 of Pl. xxxvi it is shown that the 'Stiel des Kernes' is penetrating the canal. On the formation of the egg and the significance of the micropyle he writes as follows :

'Eine der Zellen des einfachen glatten Epithels vergrößert sich und hebt dabei die anliegenden Zellen etwas mit in die Höhe. In diesem Stadium scheint das Ei lediglich aus einem Keimbläschen mit sehr geringer Dottermasse zu bestehen. Wie schon vorher die einzelnen Zellen des Epithels miteinander zusammenhängen, so bleibt auch jetzt noch die Eizelle in intimer Verbindung mit den nächstliegenden Epithelzellen, welche sie bei stetem Wachstum mehr und mehr mit sich in die Höhe zieht. . . die Mikropyle ist allerdings ein Stigma, nämlich die bis zur völligen Reife bestehende Verbindungsstelle mit den zur Eihaut umgewandelten Epithelzellen, und der Stiel, an welchem die Eier hängen, erklärt sich auf die einfachste Weise durch allmähliges Auswachsen und Abtrennen von der inneren Follikelhaut' (pp. 144-5).

I could not find the youngest stage of eggs showing the relationship with the follicular epithelium, as figured by Semper (fig. 10, *a, b, c*). But, as stated above, this peculiar structure develops quite late, apparently simultaneously with the appearance of the gelatinous coating. I feel justified in thinking that this has something to do with future changes of the egg, above all with the maturation, and is not a mere vestige of the attached part of the egg. Hamann (15, pp. 88-9, fig. 3) observed a similar structure in *H. tubulosa*. He noticed a cytoplasmic cord, which breaks through the transparent albuminous layer, ending in a round nucleus-like body outside the follicular epithelium. In my case it was different in that the rounded end always lay inside the epithelium. I could find no trace in any of my specimens of the peculiar feature observed by Mortensen (32, p. 715, Pl. xxxi, fig. 22) in *C. glacialis*, viz. that the

chromatic substance gathered on one side of the egg in the form of a dish.

Male Gonad.—I am unable to give any detailed account of the male gonad. In the specimens collected during March I found spermatogonia and spermatocytes near the germinal epithelium, while the central part of the tube was filled with unripe spermatozoa or probably spermatids. In the breeding season the internal space of the tube is filled with ripe spermatozoa, only a thin layer of larger cells—probably spermatocytes—being found near the germinal epithelium. Radiating bunches of spermatozoa, termed by Mortensen Spermatogenmae, were found in *C. glacialis* (32, p. 715, Pl. xxxi, fig. 23) and were also observed in *C. ijimai*.

Genital Papilla.—The genital papilla is situated immediately behind the tentacular crown along the mid-dorsal line. A very singular case is noticed by Ludwig (20, 1887, p. 1233), who observed that in *C. crocea* the papilla is located far backwards, in extreme cases 8 mm. back and far from the tentacular crown in an individual, which measured 40 mm. in length, and 8.5 mm. in another individual, 42 mm. long; that is one-fifth or more of the body length.

But a more noteworthy fact about the genital papilla was discovered by Edwards (13, 1910, pp. 338-9, Pl. xiii, figs. 2-5). He found in *C. frondosa* that the male genital papilla is subdivided into from four to thirty or more parts with a general average of ten, each branch ending in a terminal pore. The papilla in females is usually simple, ending in a single pore, but sometimes, though rarely, two or five pores may be met with. Further, the same author (14, 1910, pp. 599-608, Pl. xix, fig. 1) proved the presence of a similar feature in such allied species as *C. californica*, *C. miniata*, and *C. fallax*.

C. echinata offers another example of the same thing. In a male specimen which I examined the genital pores were at least fifty in number, while in females there were from five to about twenty-five pores. Previous to branching, the end of the genital duct is dilated into a wide cavity just below the cluster of minute papillae. This cavity, as well as the branched canals, is lined with a ciliated epithelium followed by a thin

layer of connective tissue and a layer of loose irregularly arranged muscular fibres.

6. SPAWNING.

The late Professor Mitsukuri observed on one occasion that the spawning had begun at 5 p.m., and in another that it had continued from 7.30 to 10.30 p.m. My experience is as follows :

At 5 p.m. on July 25, 1916, several specimens were brought to the Station, and soon after that I found two individuals emitting spermatic fluid. Half an hour afterwards, at 5.30, another individual began to lay eggs. In the morning of August 1 of the same year some few specimens were got and brought into the Station at 1.15 p.m. Soon afterwards two individuals were found emitting sperm, but in this case no laying of eggs was seen to follow. At about 2.30 p.m. of the same day plenty of large specimens were brought in. Here the emission of sperm by a male was found to begin at 5.25 p.m., and a female which was lying about 5 cm. distant from the former began to shed eggs at 5.40, which continued till about 6.30 p.m. In the specimens of the same lot kept in another jar the emission of sperm by one began at 4.50 p.m. followed by several others, but no shedding of eggs took place here.

These specimens were all quite big, and later examination showed that all of them were sexually ripe and contained spermatozoa or eggs in abundance. In the individuals which missed the chance of shedding genital elements, I noticed that it never took place the next evening or at any later time. Even exposing the animals in a warm sunny place with very little sea-water, or putting them in the dark, could not cause them to spawn. According to Mitsukuri (*ante*, p. 174) unclean water makes the animal lay eggs.

The male, while emitting spermatic fluid, stretched out its tentacles half-way and kept them very quiet. The spermatic duct could be seen through the body-wall as a white streak which appeared to perform a peristaltic movement. In consequence of the subdivision of the spermatic duct at the genital papilla, that white streak could be seen divided into five or six

branches, and a white thread-like spermatic fluid flowed out from each. Being heavier than sea-water, the spermatic fluid sank down as a milky white cloud on to the bottom of the vessel.

In females the shedding of eggs seemed to be accompanied by no waving of tentacles, but eggs were thrown out intermittently on the hinder aspect. The egg is much heavier than sea-water and very soon sinks to the bottom.

According to Kowalewsky (17, p. 1), who put about fifty freshly-caught individuals of *C. kirchsbergii* into a large vessel through which fresh sea-water flowed, the emission of sperm by males occurred within two hours. The spermatic fluid formed a white thread streaming out of a pore situated between tentacles, and was then stirred up by the waving movement of the latter. The emission of sperm lasted for about an hour, and in the next hour a female lying near began to shed eggs. He seems to have believed that the egg was fertilized inside the mother's body and was expelled through the pore in the body-wall, and that in a viviparous form, *Phyllophorus urna*, this pore serves as a birth-pore. Selenka (45, p. 166) observed no waving of tentacles in the male *C. planici* while emitting sperm, and according to him the females which began to shed eggs as soon as the males emitted sperm moved their tentacles very actively. Des Arts (2, 1910, p. 3) put a great number of *C. frondosa* into an aquarium, and the first night saw the laying of eggs which were soon fertilized. According to Newth (36, p. 633) the spawning of *C. normani* takes place in the night, and generally near midnight. On one occasion males and females lying together in the same tank began to spawn within a few minutes of one another. Isolated individuals of both sexes are said to have spawned too, but he was never successful in fertilizing the egg so shed by adding sperm suspension, and I cannot help doubting whether he was careful to ascertain that the females really spawned without being stimulated by spermatic fluid.

Of other pedate holothurians records are given by Selenka (45, p. 157) on *Holothuria tubulosa*, and by Edwards (12, p. 212) on *H. floridana*. Among some dozens of big

specimens of *H. tubulosa* kept in a large box, the males emitted sperm in the form of long white threads at intervals of two to ten minutes. After some hours fertilized eggs were found on the bottom of the box. Edwards adopted Slenka's live-box method and obtained fertilized eggs within four to ten hours.

7. NEWLY-SHED EGGS.

The egg is slightly flattened, especially so on the side of the animal pole (Pl. 8, fig. 4), as is known to be the case in *C. normani* from Newth's observation (36, p. 633). Along the axis through the poles it measures about 300–35 μ , and the greatest diameter as measured along the equatorial plane is about 340–400 μ , most commonly 400 μ . Externally the egg is covered with a radially striated gelatinous layer which is 50–72 μ thick. At the centre of the more flattened surface, the animal pole, the jelly canal can be distinctly seen. The egg is heavier than sea-water.

According to Kowalewsky (17, pp. 2, 6) the egg of *C. kirchsbergii* is opaque with a greenish yolk, and is heavier than sea-water. The egg of *C. planci* is said to be four to five times larger than that of the former species, and, according to Slenka (45, p. 167), it is lighter than sea-water and floats immediately below the surface of the water. The egg of *C. frondosa* is, as observed by Des Arts (2, p. 3), intransparent and of a red colour, with a distinct micropyle. Newth (36, p. 633) observed that the egg of *C. normani* tends to float with its animal pole directed upwards, and though no definite micropyle could be found the 'umbilicus' of the follicle seemed to act instead.

Remarkable records of large eggs are known among deep-sea forms, e.g. *Eunypniastes eximia* has an ovarian egg of 3.0–3.5 mm. diameter, and in both *Benthodytes gotoi* and *Euphronides depressa* the ovarian egg measures 2.5 mm. in diameter (Ohshima, 38, 1915, p. 214). Besides these cases, large eggs are met with in Cucumariidae, especially in those forms which are accustomed to care for their brood.

The following table shows the sizes of eggs observed in the family by various writers :

TABLE I.

Species.	Diameter of egg (mm.).		Observer.
	Ovarian Egg.	Egg newly shed or found in brood-pouch.	
<i>Cucumaria parva</i>	0.2	—	Ludwig (23, 1898)
<i>C. echinata</i>	—	0.44	Ohshima (40, 1918)
<i>C. frondosa</i>	—	0.46	Des Arts (2, 1910)
<i>Psolus granulatus</i>	—	0.5	Vaney (48, 1906)
<i>Cucumaria ijimai</i>	0.5-0.55	—	Ohshima (38, 1915)
<i>C. crocea</i>	0.6-0.65	0.7	Ludwig (20, 1887 ; 23, 1898)
<i>C. lamperti</i>	0.8	—	Ohshima (38, 1915)
<i>C. glacialis</i>	—	1.0	Mortensen (32, 1894)
<i>C. lateralis</i>	—	1.0	Vaney (48, 1906)
<i>C. curata</i>	—	1.0	Cowles (10, 1907)
<i>C. laevigata</i>	1.0	—	Lampert (18, 1889)
”	—	1.34-1.5	Ackermann (1, 1901)
<i>Thyone imbricata</i>	1.2	—	Ohshima (38, 1915)

Among the twelve species in the table, there are only two which have no brooding habit and lay eggs freely in water, namely *C. echinata* and *C. frondosa*; all the others have the brooding habit.

Maturation.—Examination of sections of the egg fixed immediately after being shed show that the egg has just extruded the first polar body (Pl. 8, fig. 4, *pb*), and that the second maturation spindle (*ps*) can be seen orientated either obliquely or vertically with reference to the circumference of the egg, while the sperm head (*sp*) has in most cases just entered. When the spindle is perpendicular to the surface a conical cytoplasmic process is formed projecting into the canal through the jelly, through which the first polar body may have been expelled. According to Boveri (4, 1901, p. 147) the canal through the jelly of the egg of a sea-urchin, *Strongylocentrotus lividus*, is widened at the maturation period and serves as the way through which the polar bodies are given out. Selenka (45, p. 167) noticed for the first time a polar body in the egg of *C. planici* and described it as ‘*der Koth des Eies*’. The egg of *C. normani* when taken from among

the tentacles of the mother is, according to Newth (36, p. 633), undergoing or has just completed the second maturation division. In the egg of *Holothuria floridana* Edwards (12) saw three polar bodies, one of which was remarkably larger than the others.

Fertilization.—The sperm head is still minute and stains intensely; it measures about 2.5μ in diameter.¹ It is found situated rather peripherally and quite distant from the animal pole, and rather near the equator (Pl. 8, fig. 4, *sp*), so that it is doubtful whether it is at the animal pole that the spermatozoon penetrates into the egg. It is highly probable that the spermatozoon enters the egg after, or at the same time as, the protrusion of the first polar body. In some sections the sperm nucleus is seen approaching the centre of the egg and becoming somewhat vesicular. In an egg fixed at fifty minutes after being shed the sperm nucleus is found lying close to the egg nucleus; it is of the same size as the latter and encircled with astral rays.

8. CLEAVAGE.

Among the eggs fixed fifty minutes after being shed were found some showing the first cleavage spindle lying horizontally at the centre of the egg. Thus the first cleavage seems to begin in about an hour. The amount of the material which I was fortunate to rear was so limited that, from fear of destroying the whole culture or in any case of losing much before any further development could be observed, I was unable to examine closely the living embryos during cleavage, &c. The following statements are given from preserved materials.

The eggs fixed within two and a half to three hours after being shed show various stages between four-cell and thirty-two-cell stages.

Four-Cell stage.—The blastomeres are equal in size, elongated along the egg-axis, and flattened or even slightly concave on the axial surface. Usually the blastomeres inter-

¹ The sperm head before entering the egg measures about 2μ in diameter.

lock, i. e. a pair situated diagonally do not lie parallel to each other but their ends approach at one pole, while the other pair approach at the other pole. In an extreme case, these two pairs come to lie in different planes, one pair being high above the other.

Eight-Cell stage.—In consequence of the interlocking of the blastomeres in the preceding stage, the two tiers of blastomeres in the eight-cell stage tend to lie shifting 45° above the other. Much irregularity in regard to the size and position of blastomeres is often met with.

Sixteen-Cell stage.—Here eight blastomeres in a tier lie above the other set consisting of eight. Very frequently, however, each tier shows a zigzag arrangement, thus the alternating four are slightly above the remaining four, and in an extreme case they form a tier of themselves at each pole.

Thirty-two-Cell stage.—Now the embryo is globular in shape leaving a spacious blastocoele inside it. In the most regular cases there are four cells in a tier on each pole, and between these there are three tiers of eight cells arranged in zigzag rows. No remarkable difference in size among the blastomeres can usually be discerned.

Above Sixty-four-Cell stage.—Now it is hardly possible to recognize any special arrangement of blastomeres. Hereafter those at and near the vegetative pole are found to be a little larger than those of the opposite pole. No coagulable matter is as yet found in the blastocoele. The blastula is found still wrapped within the egg-membrane.

According to Kowalewsky (17), Selenka (45), Edwards (12), and Des Arts (2), the cleavage of the eggs of *C. kirchbergii*, *C. planici*, *C. frondosa*, *Holothuria tubulosa*, and *H. floridana* is total and equal or approximately equal. Selenka noticed that in *C. planici* the difference of size among blastomeres is evident only after the thirty-two-cell stage, and that the cleavage ends at the beginning of the second day. Edwards stated that in *H. floridana* the four-cell stage is reached within three hours and the sixteen-cell stage within four hours. Des Arts observed in the egg

of *C. frondosa* various regular stages of cleavage on the second day. In *C. normani* and *C. saxicola*, as examined by Newth (36), the cleavage is not absolutely regular, in that the four blastomeres may rearrange themselves diagonally, and no orderly scheme could be detected in the cleavage later than the sixteen-cell stage. Only in a few individuals of the latter-named species perfect symmetry up to the sixteen-cell stage was met with. A very curious feature is seen in *C. glacialis* as reported by Mortensen (32, pp. 722-3). As a remarkable exception among echinoderms, the cleavage here is said to be superficial in that the divided nuclei migrate towards the periphery, increasing in size, and at last there is formed an epithelium, each nucleus being separated by a cell wall.

9. BLASTULA.

The blastula when free from egg-membrane floats at about the middle layer of the water, rotating actively by means of cilia. Its diameter as measured in life is about 335 μ . Though I was unable to observe its emergence from the egg-membrane, the presence of a wrinkled stage inside the membrane is hardly conceivable in view of the fact that no remarkable increase in size of the free-swimming blastula as compared with the embryonic blastula is to be found. The wall consists of a layer of very high slender cells, the vegetative pole being indicated by a thicker wall. In the blastocoele a coagulable fluid now appears, known as blastocoele jelly or 'Gallertkern' (Pl. 8, figs. 5, 6, *bj*), which increases in density with the growth of the embryo.

The blastula of *C. kirchsbergii* is said to be still covered with egg-membrane (Kowalewsky, 17). In *C. planci* the blastula is formed at the end of the first day (Ludwig, 22, p. 605) or in ten hours (Kowalewsky, 17, p. 3), and the cleavage ends early on the second day (Selenka, 45, p. 168). According to Selenka cilia arise here and there at the end of the first day, and when the cleavage is ended every cell is beset with a cilium; the embryo then gets out of the egg-membrane, and swims usually near the surface of the water. During the course of twelve hours the

blastula diminishes in size by one-fifth of its diameter, and the internal cavity becomes filled with blastocoele jelly. Ludwig (22, p. 605) denied the diminution of size in the blastula. For my part, I should think an increase of size would seem more probable in such a form where the blastula is wrinkled while inside the egg-membrane. Des Arts (2, p. 5) observed that in *C. frondosa* the blastula is formed on the third day, and that on the fourth day the cells are so multiplied that many folds appear on the surface and an irregularly formed internal cavity makes its appearance. As late as on the sixth day it is still covered with egg-membrane, but it then acquires cilia and rotates actively inside the membrane. On the seventh day it emerges from the membrane and is then $405\ \mu$ in diameter, and on the next day a thickening at the vegetative pole occurs. The same author gives further the results of the influence of the temperature upon the embryo. Besides the syncytium-formation which usually results by its being put in a warm place, the blastula, being accelerated by warmth, begins to rotate on the fifth day, and on the next day it casts off the membrane and the vegetative pole thickens. The discrepancy found between my culture and those of Mitsukuri with regard to the growth-rate, as will be stated later on, seems to be due largely, if not exclusively, to the influence of temperature. I cannot therefore lay much stress upon the time-record.

Similarly wrinkled blastulae are reported by Newth (36, p. 633) in *C. normani* and *C. saxicola*. In these species the morula is solid, and the blastocoele first appears during the formation of a wrinkled blastula. At the latter stage cilia appear and the embryo soon emerges from the egg-membrane and begins to rotate slowly at the bottom. The rotation in *C. normani* is counter-clockwise in direction as seen from above, while in *C. saxicola* it is clockwise. The wrinkled surface smoothes out before invagination occurs. According to Selenka (45, p. 160) the blastula of *H. tubulosa* acquires cilia near the end of cleavage, and at the twentieth hour it comes out of the membrane. The blastula of *H. floridana*

is, as observed by Edwards (12, p. 213), reached at the fourteenth hour.

Before invagination begins mesenchyme cells are formed by the active proliferation of the cells at the vegetative pole (Pl. 8, fig. 5). Having become free from the wall, these cells wander into the blastocoele, some lying attached to the wall near the animal pole (Pl. 8, fig. 6, *me*).

The mesenchyme-formation begins, according to the species, either before or after the invagination, or sometimes at the same time as the latter. In *C. frondosa* and *C. echinata* the mesenchyme-formation precedes the invagination. The same is true for *C. planici* in normal cases (Selenka, 45) but it may occur afterwards (Ludwig, 22). In *H. tubulosa* and *H. floridana* both the processes occur at the same time, while in Synaptids the mesenchyme cells are formed from the tip of the already formed archenteron. Ludwig (21, 1889-92, p. 258) noticed this fact and concluded that these differences are proportional to the rapidity of development. Thus in a form whose development is rapid mesenchyme is formed later, and vice versa. I may point out further that in those forms where the mesenchyme-formation takes place early the cells are generally very numerous and they readily fill up the blastocoele, while in those where invagination precedes the mesenchyme-formation the cells are generally few.

As to the origin of the mesenchyme Ludwig (21, p. 258) surmised that some mesenchyme cells may arise from the blastoderm in other places than that where the future endoderm is situated, and from his study in *C. planici* (22, p. 605) he claimed to have proved this statement. His view could not be confirmed by Newth (36, p. 635), while Clark (7, p. 61), in his observations on *Synaptula hydriformis*, felt 'no hesitation in affirming that the mesenchyme arises exclusively from the endodermal cells'. It is highly probable that Ludwig saw those cells attached to the future ectoderm, as I have mentioned above. I could not, however, find any positive evidence to support his view, and in contrast to the vegetative part where many mitotic figures

are to be met with, no such thing is found in the ectoderm. Newth observed some enucleated cytoplasmic droplets attached to the ectoderm. All I have seen were nucleated cells showing no notable difference from other mesenchyme cells suspended in the jelly. However, I cannot deny the rôle that the ectoderm plays in mesenchyme-formation in a later stage of the gastrula, followed by the appearance of stomodaeum, as will be described below.

Selenka (45, pp. 160-1, 168) observed some peculiar cells consisting partly of those detached from the blastoderm and partly of those which arose from subdivisions of the former, and called them 'Mesodermkeim'. Every subsequent observer, however, denies their presence.

10. GASTRULA.

Invagination begins early in the morning of the next day, i.e. about at the fifteenth hour. The larva gradually increases in length in accordance with the growth of the invaginated archenteron and the multiplication of mesenchyme cells. It swims with the apical end forwards, at the same time rotating around its longitudinal axis. Cilia usually beat towards the oral pole. According to Ludwig (22, p. 605) the gastrula of *C. planci* is complete at the end of the second day, while Des Arts (2, p. 8) records that in *C. frondosa* the gastrula is formed as late as on the tenth day. Newth (36, p. 633) noticed in *C. saxicola* that the direction of rotation mostly changes, and at the gastrulation is the reverse of that seen in the blastula.

The invaginated pit is beset with especially long marked cilia (Pl. 8, figs. 6, 7, *c*), which remain forming a bundle attached to the end of the archenteron for some period, still being visible even when a slight twisting has occurred in the archenteron (Pl. 8, fig. 8, *c*). The cells of the archenteron increase very actively, which fact is shown by many mitotic figures lying always near the surface and parallel to it (Pl. 8, fig. 7). The top of the archenteron shows no definite cell boundaries on the side towards the blastocoel; it here assumes the

appearance of a syncytium. Mitotic figures are found here and, as a result of rapid proliferation, the cells of the distal part detach themselves and move into the blastocoel. These detached cells continue to multiply after being free in the blastocoel. While the free mesenchyme cells are amoeboid in shape, the dividing ones are readily distinguishable by their rounded shape (Pl. 8, fig. 10).

While rapidly increasing in body length, no mitotic figures are found in the ectoderm. The cells here seem simply to decrease in height and to extend in surface. It is thickest at the hind end near the blastopore, gradually thinning out as it approaches the apical end. The nuclei lie near the internal surface in the hinder half, while in the apical half they are nearer to the outer surface. Only in abnormal embryos, which grow to an enormous size without developing beyond the gastrula, were many mitotic figures found in the ectoderm.

When the gastrula reaches its full length the archenteron almost exceeds half the length of the whole body continuing active cell-division. Selenka (45, p. 164) noticed that the archenteron lies in *H. tubulosa* near the future ventral side, and Ludwig (22, p. 605) also found in *C. planici* that it bends slightly ventrad. I have noticed no such feature in the case of *C. echinata*. At about this stage the archenteron begins to flatten in the anterior portion, and then an unequal growth of the wall occurs, resulting in the characteristic twisting of the hind part.

The above-mentioned bundle of long cilia at the bottom of the archenteron remains until about this stage. It then seems to disappear, and in the same place the archenteric wall now begins to bud off cells into the lumen of the archenteron (Pl. 8, fig. 9, *bl*). The cells thus liberated into the archenteron, sometimes called 'blood corpuscles', vary in amount according to different individuals, in some being tolerably numerous while there are none at all in others.

Archenteron.—The archenteron of the fully developed gastrula is very characteristically twisted in the sinistrorse direction, and may be described in three parts: the flat expanded

free end, the transverse ring-shaped middle part, and the longitudinal tubular end opening at the blastopore (Pl. 8, figs. 8, 11, 12, ar_{1-3}).

The first part lies perpendicularly to the frontal plane (Pl. 8, fig. 11 A, ar_1), and its posterior end approaches the left side (Pl. 8, fig. 12 A, ar_1). The frontal plane is determined from the position of the stomodaeum, which soon afterwards makes its appearance. This part is round in outline, with thickened walls near the centre, thinning out towards the periphery, making the internal lumen appear usually as a slender dumb-bell shape in transverse section. This resembles the feature found by Newth in a younger gastrula of *C. normani* (36, p. 635). It is continuous at its postero-ventral end with the second part.

The second part runs transversely round the dorsal side, across the mid-dorsal line, and bends ventrad on the right side of the body, slightly turning anteriorly (Pl. 8, figs. 11 B, 12 B, ar_2). The wall is not very thick, the lumen being somewhat compressed, with the greater diameter along the body-axis.

The third part is directly continuous with the second at the ventral end of the right limb of the latter. It runs posteriorly, and is slightly oblique to the left (Pl. 8, fig. 12 A, ar_3). In transverse section it is round, containing a narrow, often almost obliterated, internal lumen (Pl. 8, fig. 11 c). The posterior end is continuous with the blastopore.

The internal surface of the archenteron is probably lined with cilia all over, though I could not demonstrate their presence in sections.

Selenka (45, p. 170) observed in *C. planici* that when the tip of the archenteron reaches the centre of the blastocoele it begins to bifurcate. The dorsal branch increases in size very rapidly, lying obliquely forward and ventrad, while the other ventrally situated branch remains short. This stage is said to have been met with on the fourth day. According to Newth (36, p. 635; Pl. 8, fig. 8) the circular flat archenteron of the gastrula of *C. normani* turns to bend in an S-shape at right angles to its plane of flattening, and the anterior flattened sac lies obliquely to the body-axis.

My own observation on the specimens of *C. planci* brought back from Naples by the late Professor Mitsukuri shows clearly that the archenteron is twisted exactly in the same manner as in *C. echinata*. It seems to me highly probable that Selenka's figure (Pl. xl, fig. 21) was obtained from a thick section, as he was apparently unable to get a good series of well-orientated sections. His figure is said to represent a sagittal section, but really it is a frontal one. From Newth's figure of a longitudinal section of a forty-fourth-hour gastrula of *C. normani* (Pl. 8, fig. 8) it is obvious that the archenteron is not simply folded in an S-shape, but is twisted in a spiral. The figure, too, is a frontal section, I believe, not a sagittal one as he supposed.

It was found by Edwards (12, p. 213) in *Holothuria floridana* that by the twenty-second hour a plug of cells grows out from the blind end of the archenteron towards the blastopore, and that by this plug the archenteron is divided into the dorsal and ventral branches. No such changes were observed by Selenka in *H. tubulosa*.

In *C. planci* the position of the blastopore changes, according to Selenka, slightly towards the future dorsal side, but according to Ludwig it is said to shift ventrad. I could not decide which of the two holds true in my case. In most cases the blastopore opens at the hind end.

Stomodaeum. The stomodaeum makes its first appearance in the quite old gastrula, where the archenteron begins to divide into hydro-enterocoele and gut (Pl. 8, figs. 13, 14 A, *st*). It is preceded by a thickening of ectoderm on the ventral side at about the middle of the body (Pl. 8, fig. 11 A, *sy*). This is partly due to a sinking down of the ectodermal cells and partly to an accumulation of the multiplying mesenchyme cells. Here a syncytium is formed, the internal surface of the ectoderm not being a definite one, touching the ventral edge of the flattened part of the archenteron. The surface of the latter is still clearly cut, no mitotic figures being found on this side.

The stomodaeal depression then comes in sight a little on the left side of the plane in which the flattened part of the archen-

teron has been lying (Pl. 8, fig. 14 A. *st*). These changes very much resemble the mesenchyme-formation and the invagination process occurring in the late blastula. Only in this place does Ludwig's opinion, that the ectoderm shares in the mesenchyme-formation, seem to be true.

This author observed in the third-day larva of *C. planici* that the stomodaeum appeared on the ventral side immediately behind the pre-oral hood (22, p. 606). According to Newth (36, p. 634), the stomodaeum appears in *C. saxicola* and *C. normani* in forty-eight hours, i.e. on the middle of the third day, as a crescentic invagination at the junction of the opaque and less opaque regions. The horns of the crescent extend backwards and ultimately fuse up and the enclosed area sinks in. It lies very obviously to the left of the mid-ventral line as determined by the pedicels. Similarly a crescentic depression appears in the second-day embryo of *H. floridana*, according to Edwards (12, p. 213), but it gradually deepens and straightens, growing out to either side until it extends entirely across the ventral surface. The plane in which the groove lies is at an angle of 50° with the sagittal plane of the adult holothurian.

11. DIPLEURULA.

Under the term 'dipleurula' I mean the stage which connects the gastrula with the barrel-shaped larva or doliolaria. This stage is characterized by remarkable changes occurring in the archenteron accompanied by a rapid increase of the mesenchyme cells. As seen from the exterior the larva has become slightly shorter than in the foregoing stage, the stomodaeum has appeared, and it differs from the next stage in having no ciliary bands. This stage is passed during the thirtieth to fortieth hours, i.e. from the end of the second day till early in the morning of the third.

Ludwig (21, pp. 274-5) suggested that there might be a stage, reminding us of the auricularia, during the changes which take place between the gastrula and the barrel-shaped stage. In his study of *C. planici* (22, p. 606) he pointed out the fact that the buccal cavity has at the beginning a garland-

shaped thickening on its edge, which he believed to be homologous with the ciliary band of the auricularia. I was unable to verify Ludwig's opinion, but the stage which I call dipleurula is homologous with the auricularia in respect to the arrangement of the internal vesicles.

The arrangement of the ciliary bands as well as the degrees of development of the alimentary tract cannot, I believe, help us in discussing homologies among different forms, because they vary in degree according to different modes of living. In the free-living auricularia of *Labidoplax digitata* the alimentary canal is well differentiated into fore-, mid-, and hind-gut, and the ciliary band is typically developed, as is well known from the records of many observers. The embryo of *Synaptula hydriformis* developing inside the mother's body-cavity retains an elliptical shape of body, showing no trace of any ciliary band, the gut being quite rudimentary (Clark, 7, p. 62). Another viviparous form, *Chiridota rotifera*, shows an intermediate feature between the above two (Clark, 9, p. 501).

The division of the twisted archenteron in the old gastrula occurs first at the postero-ventral end of the second part, where a solid cell-mass with obliterated lumen connects the divided portions for a while (Pl. 8, fig. 13). The larger vesicular portion, consisting of the first and second parts of the archenteron is now to be called hydro-enterocoele or vaso-peritoneal vesicle, while the smaller one which was the third part is the future gut. The latter has a very narrow lumen, in most cases being still continuous with the exterior through the blastopore.

The next change occurring in the larva is the displacement, change of shape, and division of the hydro-enterocoele, and the enormous multiplication of the mesenchyme cells which fill up the blastocoele, so that no external examination of the internal structure on clarified material is now possible.

The anterior part of the hydro-enterocoele, which in the late gastrula was concave on the right side (Pl. 8, fig. 14 A, *ar*₁), now moves round to the right across the dorsal side and becomes narrow in breadth (Pl. 8, fig. 16 A, *hy*). The posterior part

of the same vesicle, on the contrary, moves to the left through the dorsal side (fig. 16 c, *en*), and, as the two parts thus move in opposite directions, they gradually begin to be cut off from each other a little on the left side (Pl. 8, fig. 15).

The anterior part, which will give rise to hydrocoele (*hy*), gives out from the postero-dorsal margin obliquely backwards a conical process which finally unites with the dorsal ectoderm (*pc*). This is the rudiment of the pore-canal. The posterior part, which is the future enterocoele (*en*), is a little smaller than the anterior part and lies on the left side, extending round the body-axis and stretching from the antero-ventral to the postero-dorsal side.

The walls of the hydrocoele and enterocoele consist of a single layer of cells, clearly distinct from the free mesenchyme cells, and the latter do not yet attach themselves to the surface of the former.

The first observer who traced the fate of the archenteron was Selenka (45, p. 170). He noticed that in *C. plani* the archenteron bifurcated at the top, and that the dorsal branch increased rapidly in size, bending obliquely antero-ventrad, and at last becomes separated as a vaso-peritoneal vesicle from the other branch, which latter was stunted and later gave rise to the gut ('Körperdarm'). In my opinion, his two branches are a complete vaso-peritoneal vesicle, and he seems to have overlooked the separation of the gut from that vesicle. He further stated that after the separation of the two vesicles the vaso-peritoneal vesicle shifted to the left side of the gut, while the latter rapidly grew forwards and at last united with a ventral invagination ('Munddarm'). He is right in saying that the first part then lies on the left side, but that the gut breaks through to the stomodaeum is improbable at such an early stage, and moreover, the part which he called 'Körperdarm' is, I think, to be identified with the enterocoele, which should never have any communication with the stomodaeum at all. A careful comparison of his figs. 21, Pl. xi, and 22 B, Pl. xii, leads one to conclude that the part he denoted P (enterocoele) in the fig. 22 B is derived from that part denoted B in the fig. 21.

His fig. 22 B resembles very much what I observed in *C. echinata* in a corresponding stage.

Ludwig (22, p. 605) observed in the third-day larva of *C. planici* that the hydro-enterocoel had separated from the rest of the archenteron, and again divided into the hydrocoel and two enterocoel vesicles. Some larvae were somewhat younger, and in them the hydro-enterocoel was still in connexion with the archenteron. In these statements he seems to have been unable to give the time and sequence of the separations of those vesicles. Selenka was of the opinion that the hydro-enterocoel, at first as long as it is connected with the gut, lies on the dorsal side of the latter, but shifts to the left side about the time when the separation sets in. Ludwig observed, contrary to Selenka, that it had been lying on this side from the beginning. I agree with Ludwig on this point.

Newth (36, p. 635) could not be sure about the breaking off of the archenteron in *C. saxicola* and *C. normani*, being only able to say that the water-vascular system, the posterior (perivisceral) coelom, and the gut are separated from successive regions of the archenteron in the order named, beginning at the anterior end. In *C. normani* the separation of the enterocoel was complete by the middle of the third day, though in some individuals the hydro-enterocoel connexion was not then broken.

The hydrocoel then increases in breadth again, stretching round the right side of the body, and its free anterior margin begins to divide into lobes, which are indistinct at first but rapidly become distinct processes. These changes as well as those of the enterocoel to be mentioned below vary very much according to different individuals. The following statements seem, however, to represent what is most frequently met with.

Hydrocoel.—In the beginning three lobes are formed (Pl. 9, fig. 17, *hy*). The first is narrow, situated at the left anterior corner, the second is broad, formed of the greater part of the anterior margin of the hydrocoel, and the third is again narrow, situated on the right ventral edge of the hydrocoel

directed transversely. The second broad part then divides into three lobes almost equal in breadth, while another lobe arises on the left edge, behind the first lobe (Pl. 9, fig. 19). Thus the hydrocoele is now fan-shaped, with a narrow conical process directed postero-dorsally, which is the future pore-canal, and an expanded anterior margin, wavy with six lobes as just described. From that transverse lobe, at first numbered third, is formed the mid-ventral radial canal (*mr*), while the other five lobes are rudiments of the five primary tentacles (*t*). Except the mid-ventral one no other radial canals appear at this stage. The pore-canal opens to the exterior through the dorsal pore about the end of these changes (*dp*).

Kowalewsky (17, p. 4) and Selenka (45, p. 171) are of the same opinion that, in *C. kirchbergii* and *C. planci* respectively, there are first formed only three tentacles situated dorsally, and the remaining two appear after the hydrocoele ring has closed. Ludwig (22, p. 608) found, on the contrary, that in *C. planci* the five primary tentacles appear simultaneously as outgrowths of the radial canals. This divergence in view from other observers results probably from the fact that he originally dealt with eighth-day larvae without examining any earlier stages. I agree with Kowalewsky and Selenka in assuming the appearance of the tentacles to be earlier than that of the radial canals, but those three lobes which first appeared are, in my opinion, not the dorsal three of the primary tentacles. Ludwig's account of the early features of the hydrocoele is very incomplete owing to the lack of any intermediate stages. According to him the hydrocoele is of an irregular horseshoe shape, whose arched part lies dorsally, the right limb is short, stretching obliquely antero-ventrad ('nach vorn und unten'), and the left limb is longer, stretching postero-ventrad ('nach unten und hinten'). I never observed such a condition, and am convinced that he was in error in these statements, from which he drew an incorrect conclusion that the hydrocoele ring probably closed on the right side.

Enterocele.—Soon after being separated from the hydrocoele the enterocele divides into two vesicles, one larger and

antero-ventral in position, the other smaller and on the left dorsal side stretching posteriorly (Pl. 9, figs. 17, 18, *le, re*). The former corresponds with the left enterocoel of other echinoderms, while the latter, although situated at first on the left dorsal side, is the right enterocoel. Selenka (45, p. 171) noticed in *C. planici* that the peritoneal vesicle (enterocoel) divides, immediately after being separated from the vascular vesicle (hydrocoel), into two ellipsoid vesicles lying on the right and left sides of the gut respectively. Likewise in *H. tubulosa* the enterocoel which stretches behind and below the gut divides into two vesicles which lie symmetrically on each side of the gut.

Stomodaeum.—The stomodaeum is formed by an encircling of the slit-like depression and a sinking down of the included area. It contains a thin lumen, extending parallel to and below the external surface, which opens through a narrow orifice to the exterior (Pl. 8, fig. 16 A; Pl. 9, fig. 18 A, *st*). The syncytium (*sy*) extending over the stomodaeum grows between the hydrocoel and enterocoels to form a solid cell-mass running backwards to join with the gut. The gut is of a single layer of cells but very thick, leaving a narrow lumen inside (Pl. 9, fig. 18 B, *g*).

12. DOLIOLARIA.

The doliolaria, or barrel-shaped stage, is reached about at the fortieth to the fiftieth hour, i. e. on the third day. This is characterized by the acquisition of three transverse ciliary bands on the posterior half of the body, the appearance of rudiments of the pedicels, and the further development of the five primary tentacles and radial canals. This stage lasts until the fourth day or even the eighth day or more.¹

The larva measures above 500 μ in length, and swims usually immediately beneath the surface of the water, being either vertical or oblique in position. Cilia beat usually towards the posterior

¹ My own culture showed no evidence of changing into the pentaetula-stage even on the eighth day, when I had to leave Misaki and could not follow any further changes.

end but often reverse, which latter movement makes the larva sink to the deeper part of the water. Besides these two kinds of locomotion, rotation around the body-axis is observed at the same time. In no case is the pre-oral hood directed downwards.

Although no marked change is visible externally, the latter half of the stage had better be treated under a distinct heading, *Metadoliolaria*, owing to its internal changes. Here in the present chapter I will confine myself to the earlier part, *doliolaria* in the narrow sense.

In the corresponding stage of *C. frondosa*, Danielssen and Koren (11) found that rudiments of the tentacles appear on the tenth day and a pair of the primary pedicels on the twentieth. Des Arts (2, p. 9) observed in the same species that the larva measures on the fifteenth day 510μ by 375μ , and that the tentacles are visible in section on the twenty-first day, but are observable externally so late as on the twenty-fourth day, and the pedicels make their first appearance on the thirty-seventh day. The internal structure of the *doliolaria* of *C. kirchbergii* was described and figured by Kowalewsky (17, fig. 12). The same author gave an external view of the larva of *C. planci* (figs. 16, 17), while Selenka (45) and Ludwig (22) made much closer observations. From the observations of Newth (36), we gather that the corresponding stage in *C. saxicola* and *C. normani* is not distinguishable externally from lack of the ciliary bands which are so characteristic of the stage in other species.

Ciliation of the Ectoderm.—The presence of three, very rarely four, transversely-running bands of cilia is a very marked character of *doliolaria*. They seem to appear simultaneously. The most anterior band lies about on the middle of the body (Pl. 9, fig. 25, c_1), the second and third run parallel to the former and in such a way that they divide the posterior half of the body into three equal divisions, or, as is often the case, the hindmost third is a little broader than the other two (c_{2-3}). In preserved specimens the cilia are extremely difficult to make out, but they can easily be found in the living state.

The length, as roughly measured, is about 25μ . Much weaker cilia are found uniformly covering both the parts anterior to the first ciliary band and that posterior to the third. The areas lying between the bands seem to be devoid of them.

In *C. planici* the ciliary bands present are four (Kowalewsky) or very rarely five (Selenka) in number, besides the uniform ciliation all over the pre-oral hood and anal field. After the appearance of pedicels and tentacles these uniform weaker cilia disappear (Selenka, **45**, p. 172). Mortensen (**33**, pp. 23-4; Pl. i, fig. 8, *a, b, c*) could not ascertain the presence of ciliary bands in preserved specimens of doliolaria which are about 1 mm. long, of a light reddish colour, and which were found in the Southern Kattegat. He referred them to *Psolus phantapus* and suspected the presence of three ciliary bands. I am much inclined to believe that there are four bands running along the circular spaces free from calcareous bodies (fig. 8, *c*). In *C. frondosa* we have no record of ciliary bands (Danielssen and Koren, **11**; Des Arts, **2**), and in *C. kirchsbergii* the bands seem to be really absent (Kowalewsky, **17**). Doliolariae of *C. saxicola* and *C. normani* are ciliated uniformly all over as in other stages, no segregation of cilia into bands being found (Newth, **36**). The larva of *Phyllophorus urna*, too, shows no zonary distribution of cilia while actively swimming inside the mother's body-cavity (Kowalewsky, **17**, p. 7).

When examined in section, the cilia are very obscure and markedly short, due to shrinkage. The ectoderm is thickened at the band, being about twice as thick as other parts, and of a lens shape in transverse section (Pl. 9, fig. 25, c_{1-3}). The nuclei are situated near the base of the cells. As to these cells I could find no distinction between 'Wimperzellen' and 'Reservezellen' as Reimers (**43**, 1912, p. 270) did in his observations on the larva of *Labidoplax digitata*. Further, I could not clearly make out either 'Binnenfaser' or 'Basalstäbchen' as clearly figured by him (Pl. ii, figs. 5-9).

Hydrocoele.—The lobe no. 6 of the hydrocoele, as numbered from the dorsal one towards the ventrum, stretches

out ventrad, bringing together with it the lobes nos. 4 and 5, and on reaching the mid-ventral line it turns posteriorly to give rise to the mid-ventral radial canal (Pl. 9, fig. 21 A, *mv*). The other lobes except no. 1 are now differentiated into cylindrical tubes—primary tentacles (t_{2-5})—connected at the base by a rather narrow canal, which forms a horseshoe-shaped rudiment of the ring canal. The lobe no. 1 eventually gives rise both to the remaining one of the primary tentacles and to the free end of the dorsal limb of the open hydrocoele ring. It remains for a while as an inconspicuous outgrowth.

Radial Canals.—The free end of the rudimentary mid-ventral radial canal then dilates laterally to form a rhombic vesicle in ventral view, and then takes on a cross shape (Pl. 9, fig. 23 A, *mv*). The transverse branches thus formed are the primary pedicel canals (*rpc*, *lpc*), and in correspondence with each of them a rudiment of the primary pair of pedicels (*rp*, *lp*) is formed.

The four radial canals, other than the mid-ventral one, are formed comparatively late, especially the ventral pair (*rd*, *ld*, *rv*, *lv*). They arise at first as small knobs on the anterior margin of the ring canal, one in each interval of the primary tentacles. The knobs then bend outwards and immediately turn posteriorly, and they are remarkably thin as compared with the mid-ventral one.

Kowalewsky (17, p. 4) first described in *C. kirchbergii* the first appearance of the mid-ventral radial canal, which soon divided into two, pushing the body-wall outwards to form a pair of pedicels. In *C. planei* Selenka (45, p. 171) ascribed the development of the mid-ventral radial canal to too late a period, stating that it made its first appearance soon after the closure of the hydrocoele ring, and that four other radial canals and the Polian vesicle followed it. As to the fact that the four radial canals, other than the mid-ventral one, appear later than the latter, all observers are unanimous. Ludwig (22, p. 181) further noticed that among those four the ventral pair are shorter and narrower than the dorsal pair, the difference being observable throughout the life of the young; they grow to be equal much later on.

Primary Pedicels.—A pair of the primary pedicels are first indicated by circular pits formed on the ectoderm corresponding to the pedicel canals branching from the mid-ventral radial canal (Pl. 9, fig. 22 D, *p*; fig. 24 F, *lp*, *rp*). These I may call pedal pits. The formation of the pedal pits a good deal resembles that of the stomodaeum, the rudiment of the pedicel being formed by the syncytium below the pit and arising from the bottom of the pit covered by the ectoderm. Finally it projects from the pit, the latter being soon flattened out. These changes strikingly resemble those found in the primary tentacles. The pits are situated between the second and third ciliary bands and at an angle of about 40° on each side of the sagittal plane. Specimens are often found in which only one of the pair has just appeared. Of six cases of such specimens I observed all had only the left pedicel (Pl. 9, figs. 21, 22, *p*). Out of seventeen cases where both the pedicels had appeared, eight cases showed that the left pedicel lies more or less anteriorly to the right one, while seven cases were the reverse, and in the remaining two cases the two lay on the same level. Thus we can find no constant feature as to the relative position of the two primary pedicels.

According to Ludwig (22, pp. 185, 607), in *C. planei* the pedal pits appear in most cases near the end of the fourth day, and the pair of pedicel canals appear from the mid-ventral radial canal either at the end of that day or early on the next. Of the pair of pedal pits the right one always lies a little anterior to the left one, and the same holds true in *Phyllophorus urna* as observed by the same author (24, p. 97). Newth (36, p. 637) found in the third-day larvae of *C. saxicola* and *C. normani* that the posterior end of the mid-ventral radial canal formed a rhombic dilation representing the rudiments of pedicel canals. Here, he says, the left pedicel lies further forward than the right, just contrary to the feature seen in *C. planei* and *Ph. urna*.

From Edwards's observation (12, pp. 222-3) we learn that in *H. floridana* the first pedicel is unpaired and appears at the posterior end of the mid-ventral radial canal. It is said

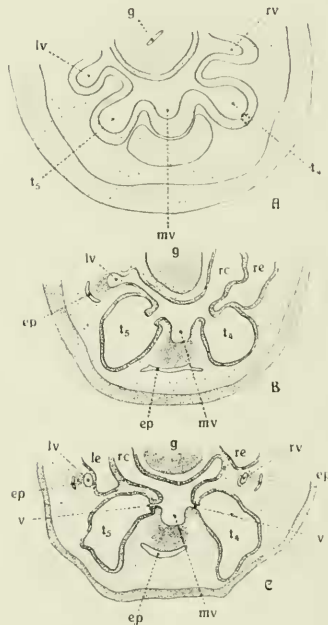
that J. Müller had observed in the young developed from 'Auricularia mit Kugeln' that the first pedicel was put forth on the right from the mid-ventral radial canal (Ludwig, 21, p. 291).

Primary Tentacles.—Now the five rudiments of primary tentacles can be referred to the respective interradius (Pl. 9, figs. 23 A, B; 24 A-D; t_{1-5}). No. 2 (t_2) lies in the mid-dorsal interradius, no. 3 (t_3) in the right dorsal, no. 4 (t_4) in the right ventral, no. 5 (t_5) in the left ventral, while the remaining one, no. 1 (t_1), which should arise from the dorsalmost lobe, and is the last to appear, is in the left dorsal interradius.

In their early stages the five primary tentacles arise directly from the ring canal, or more properly speaking, the hydrocoele differentiates into the tentacle-rudiments and the ring canal. In the course of growth a peculiar grouping of tentacles begins to appear in relation to the radial canals (Text-fig. 4. A-C). It seems very probable that the rudiments of the radial canals develop at the expense of the adjoining parts of the hydrocoele ring, attracting towards them the bases of the tentacle-rudiments, so that the latter seemingly become branches given out from the radial canals, totally independent of the ring canal. The grouping of tentacles is precisely the same as that found by Ludwig (22, pp. 183, 608) in *C. planici*, i. e. the ventral pair gather at the mid-ventral radius, that of the right dorsal interradius moves towards the right dorsal radius, and the remaining two meet with the left dorsal radial canal (Text-fig. 9 A, I, I'). Ludwig insisted upon the opinion that all the five primary tentacles appeared simultaneously as branches of the radial canals, not directly from the ring canal. One must keep in mind that Ludwig's first observations were made on the eighth-day larvae, which are quite well established doliolariae, and that in his second report he tried to trace the fact as far back as to the fourth-day larvae. Newth (36, p. 637-8), confirming Selenka's view, claims that the primary tentacles are originally interradiar in position, arising from the ring canal directly and alternating with the radial canals. He found that some individuals had shown on the third day that curious grouping

of the three dorsal tentacles, while still on the fourth day the ventral two retain their original interradial position, their bases, however, beginning to approach the mid-ventral radial canal. According to Edwards (12, p. 216) the feature is quite different in *H. floridana*. Here on the fourth day each

TEXT-FIG. 4.



Cross-sections cut at the height of the ring canal, to show the gradual displacement of primary tentacles. A. *Doliolaria*, reconstructed from several sections. B. *Metadoliolaria*. C. Still advanced *metadoliolaria*. $\times 200$. *ep* = epineural canal; *g* = gut; *le* = left enterocoele; *lv* = left ventral radial canal; *mv* = mid-ventral radial canal; *rc* = ring canal; *re* = right enterocoele; *rv* = right ventral radial canal; *t*_{4,5} = primary tentacles; *v* = ventilating apparatus.

of the mid-ventral and the left dorsal radial canals produces a tentacle on the right side, and each of the paired ventral radial canals sends out one on the dorsal side. Of these four, the former two seem to be the first to develop. The fifth appears

last on the left side of the mid-ventral radial canal (Text-fig. 9B, I, I'): this attains a size equal to the other four as late as the seventh day. Becher (3, 1908, p. 4) attributes this difference between *Cucumaria* and *Holothuria* to the higher tentacle number of the latter in the adult. This must not be rashly concluded since the feature in question has not yet been recorded in other many-tentacled forms such as *Phyllophorus*, *Pseudocucumis*, &c. Apart from the difference in the sequence of their appearance, the distribution of the primary tentacles with regard to the radii does not differ very much in *Cucumaria* and *Holothuria*. Indeed, the two agree in the circumstance that the tentacles alternate with the radial canals as pointed out by Newth (p. 639). The only notable difference is that in *Cucumaria* each of the pair lying in the dorso-lateral interradii is supplied by a tentacular canal sent ventrad from the dorso-lateral radial canal of each side, while in *Holothuria* the corresponding tentacles are supplied by branches sent dorsad from the ventro-lateral radial canals. Now, if we are right to admit that the primary tentacles all originate directly from the ring canal, but not from radial canals, such difference as found between these two genera does not seem to me so great and fundamental. As will be shown later, in *Cucumaria* the dorsal pair of radial canals grow faster than the ventral pair, so that the bases of the tentacles in question shift dorsally and at last become branches of the dorsal pair. In *Holothuria*, on the contrary, the ventral pair of radial canals, being more vigorous in growth than the dorsal pair, might have conquered in pulling together the bases of those tentacles. As a matter of fact, in *Holothuria* the ventro-lateral radial canals are in adult state generally more strongly developed than the dorsal pair, while in *Cucumaria* these two pairs differ very little. Another common feature in both cases is that two of the primary tentacles belong to the mid-ventral radial canal. It is a noteworthy fact that even in such a form as *C. echinata*, whose ventral pair of tentacles are markedly smaller than the remaining eight in the adult stage, the former are represented

in the five primary tentacles (compare Becher, 3, p. 4). It is an open question how the mid-ventral radial canal behaves in the early stages of *Sphaerothuria bitentaculata*, which possesses only eight tentacles when adult, the mid-ventral radial canal supplying no tentacles at all.

Polian Vesicle.—The Polian vesicle appears at the free end of the ventral limb of the open hydrocoele ring, directed posteriorly and lying inside the enterocoele vesicle (Pl. 9, figs. 23 A, B; 24 D; *pr*). It may often appear after the closure of the ring.

Stone-Canal.—About at the same time as the appearance of the Polian vesicle the pore-canal swells out dorsally at its middle part, and as a result of it the canal slightly bends at this point (Pl. 9, fig. 25, *as*). In transverse section this swelled part shows a very characteristic feature in that the wall of the axial side is of a very high epithelium, while along the dorsal side the wall is very thin. Bury (5, 1889, p. 427; Pl. xxxix, fig. 26) first noticed this structure in a *Cucumaria*, and considered it as a vestigial anterior enterocoele, the presence of which he had proved in auricularia. Ludwig (22, p. 609) observed it in the fifth-day larva of *C. planici* and called it the madreporic vesicle, with an assumption that it is only a secondary outgrowth. In his later paper Bury (6, 1895, pp. 53-4) insisted upon his former view, and suggested that future and closer examinations would reveal changes similar to those in auricularia, proving its origin from the enterocoele. According to Newth (36, p. 637) this enlargement occurs by an up-pushing of the antero-dorsal wall of the canal on the third day in *C. saxicola* and *C. normani*. On the next day the cells of the antero-dorsal wall of this vesicle become large and clear. This swelling up of the cells seemed to him to be a preliminary stage in the thinning out of the part as seen by Bury, Ludwig, &c. I was unable to find either the change which Bury suggested to be present or the swelling up of the cells in the early stages of this structure. The same structure has further been proved to be present in *Phyllophorus urna* by Ludwig (24, p. 98) and by Russo

(44, p. 45; Pl. iii, fig. 52), in *Holothuria floridana* by Edwards (12, p. 214), in *Cucumaria crocea* and another antarctic *Cucumaria* by MacBride (25, pp. 7, 8; 27, p. 4). The last-named author called this vesicle the axial sinus.

The distal portion of the canal, which should now properly be called the pore-canal, runs through the dorsal body-wall and opens to the exterior. The opening, or dorsal pore, is situated between the second and third ciliary bands, and is in most cases slightly on the right of the mid-dorsal line (Pl. 9, fig. 22 B, *dp*; fig. 24 E, *pe*). Ludwig (22, p. 186) also found that the pore opens on the right.

Closure of the Ring Canal.—From the fact that the rudiment of the left ventral radial canal appears on the ventral limb of the open hydrocoele ring, while that of the left dorsal radial canal belongs to the dorsal limb of the same, it is clear that the closure of the ring occurs on the left dorsal interradius. The Polian vesicle lies at first very near to the left ventral radius, but later it moves towards the middle of the dorsal interradius, which is its normal position as found in adult individuals.

As to the time and position of the closure of the ring no entirely satisfactory observations have been given. Kowalewsky (17, p. 4) and Selenka (45, p. 171) were in agreement in the opinion that the ring closed after the formation of three dorsal tentacles, while the remaining two developed from the closed ring. Ludwig (22, p. 607) observed in *C. planci* that the ring was complete at the end of the fourth day, and the closure seemed to have taken place on the right side of the body. From this incorrect view he concluded that the Polian vesicle which lay on the left dorsal interradius could not be an indication of the point of closure. Newth (37, p. 637) is quite right in concluding that in *C. saxicola* the ring closed in the left dorsal interradius on the third day, when the radii can be identified. He could not determine to which limb of the free ends of the unclosed ring the rudiment of the Polian vesicle belonged, being only able to say that it

was found as a small blunt outgrowth produced at the point of closure.

From the table on p. 215 the following features may be summarized :

1. Of the five primary tentacles the one situated in the left dorsal interradius appears last (nos. 1-4).

2. To the four tentacles and one mid-ventral radial canal the right dorsal radial canal is first added (nos. 2-4).

3. The left dorsal radial canal appears at about the same time as the appearance of the fifth tentacle, after which the right ventral radial canal follows immediately (nos. 5-7).

4. The appearance of the left ventral radial canal is still later (no. 10).

5. The appearance of the Polian vesicle in some cases precedes the closure of the ring canal (nos. 8, 9, and 12), and in others it is later (nos. 6, 11, 13, and 15).

6. The formation of the axial sinus also in some cases precedes the closure of the ring (nos. 8, 9) and in others it is later (nos. 6, 11).

7. The closure of the ring takes place in most cases after five radial canals have all appeared.

Stomodaeum.—Now the position of the stomodaeum can be determined by the establishment of the mid-ventral radial canal. It lies in front of the first ciliary band, and at about 30° to the left of the sagittal plane. The ectoderm covering the interior of the atrial cavity is pushed up by the growing tentacles, forming an epidermal covering for the latter (Pl. 9, fig. 25, *at*). The orifice is often found plugged up by the left ventral tentacle which lies nearest to the stomodaeum (Text-fig. 5, *t*₅). Newth (36, p. 634) noticed the asymmetrical position of the stomodaeum, while Ludwig (22, p. 610) observed the same fact but interpreted it erroneously. He was of the opinion that the larval symmetry plane is not coincident with that of the adult, and thought that the left ventral tentacle stands nearest to the mid-ventral line.

Simultaneously with the growth of the primary tentacles and the diminution of the pre-oral hood, the stomodaeum

TABLE II.

To show the development of the hydrocoele appendages and the time of closure of the ring canal.

In the table are indicated whether the tentacles, Polian vesicle, and axial sinus are formed (\times) or not yet (-); whether the ring canal is still open (*o*) or closed (*c*); whether the rudiments of radial canals are only buds (*b*), or directed anteriorly (*a*), or laterally outwards (*l*), or posteriorly (*p*); whether the mid-ventral radial canal ends simply in the form of a knob (*k*), or is slightly expanded into a rhombic vesicle (*r*), or has formed a pair of lateral branches, pedicel canals (*pd*).

No.	Age in days.	Ring canal.	Primary tentacles.					Radial canals.					Polian vesicle.	Axial sinus. ¹		
			1 (td.)	2 (md.)	3 (rd.)	4 (rv.)	5 (lc.)	LD.	RD.	RF.	MF.	LV.				
1	2	o.	-	\times	\times	\times	\times	-	-	-	-	-	-	-	-	-
2 ²	1.5	o.	-	\times	\times	\times	\times	-	<i>a.</i>	-	<i>k.</i>	-	-	-	-	-
3	2	o.	-	\times	\times	\times	\times	-	<i>a.</i>	-	<i>r.</i>	-	-	-	-	-
4	2	o.	-	\times	\times	\times	\times	-	<i>l.</i>	-	<i>k.</i>	-	-	-	-	-
5	2	o.	\times	\times	\times	\times	\times	<i>b.</i>	<i>a.</i>	<i>l.</i>	<i>k.</i>	-	-	-	-	-
6	2	c.	\times	\times	\times	\times	\times	<i>a.</i>	<i>l.</i>	-	<i>k.</i>	-	-	-	-	-
7	2	o.	\times	\times	\times	\times	\times	<i>l.</i>	<i>l.</i>	<i>a.</i>	<i>r.</i>	-	-	-	-	-
8 ³	2	o.	\times	\times	\times	\times	\times	<i>p.</i>	<i>p.</i>	<i>p.</i>	<i>pd.</i>	-	-	-	\times	\times
9	2	o.	\times	\times	\times	\times	\times	<i>p.</i>	<i>p.</i>	<i>p.</i>	<i>pd.</i>	-	-	-	\times	\times
10	2	?	\times	\times	\times	\times	\times	<i>p.</i>	<i>p.</i>	<i>p.</i>	<i>pd.</i>	-	-	-	\times	\times
11	2	c.	\times	\times	\times	\times	\times	<i>l.</i>	<i>l.</i>	<i>l.</i>	<i>pd.</i>	-	-	-	\times	\times
12 ⁴	2	o.	\times	\times	\times	\times	\times	<i>l.</i>	<i>l.</i>	<i>l.</i>	<i>pd.</i>	-	-	-	\times	\times
13	2	c.	\times	\times	\times	\times	\times	<i>l.</i>	<i>p.</i>	<i>l.</i>	<i>pd.</i>	-	-	-	\times	\times
14	2	c.	\times	\times	\times	\times	\times	<i>p.</i>	<i>p.</i>	<i>l.</i>	<i>pd.</i>	-	-	-	\times	\times
15	3	c.	\times	\times	\times	\times	\times	<i>p.</i>	<i>p.</i>	<i>p.</i>	<i>pd.</i>	-	-	-	\times	\times
16	3	c.	\times	\times	\times	\times	\times	<i>p.</i>	<i>p.</i>	<i>p.</i>	<i>pd.</i>	-	-	-	\times	\times

¹ The pore-canal is not yet open dorsally in no. 1, but no. 2 and all subsequent specimens have the dorsal pore open.

² This specimen is shown in Pl. 9, figs. 21, 22.

³ This specimen is shown in Pl. 9, fig. 25.

⁴ This specimen is shown in Pl. 9, figs. 23, 24.

gradually widens and at last flattens out, so that the tentacles freely protrude above the body-surface.

Alimentary Canal.—The rudiment of the gut has been growing both in length and diameter by rapid cell-division and by increase of the cells in height. Its anterior end extends beyond the ring canal by which it is encircled (Pl. 9, fig. 25, *g*), and while both ends remain solid its middle portion has a distinctly discernible flat lumen lying parallel to the frontal plane (Pl. 9, figs. 24 E, F; *g*).

Enterocoeles.—Early in the doliolaria stage, where four of the primary tentacles have become apparent, the right and left enterocoeles come into contact with each other at their free margins. Both the enterocoeles have been rapidly growing in size, extending across the median line and encircling the gut. The fusion of their ends takes place on the right side, beginning either at the anterior part or at the posterior part of the line of contact, leaving for a while an oblique incision at either end of the line (Pl. 9, figs. 21, 23; *re, le*).

The other ends of the two vesicles approach each other but are separated by a narrow interval. This intervening part gives rise to the dorsal mesentery in the end, and lies at first obliquely on the left side, beginning anteriorly near the mid-dorsal line to end near the mid-ventral line. It, however, gradually bends into an S-shape, indicating the three sections as found in the future mesentery—the first, mid-dorsal and descending section; the second, oblique and ascending section on the left; and the third, descending section running along the mid-ventral line.

I failed to find any ‘finger-like process’ as seen by Bury (6, p. 48) in Synaptids and verified by others. Though very often there appears a process on the antero-dorsal end of the enterocoele, stretching beyond the primary stone-canal to the left, I could not follow its fate, and am uncertain whether the peripharyngeal sinus originates from it or not.

The behaviour of the enterocoeles in *C. planei* was first observed by Selenka (45, p. 171), according to whom the union of the right and left vesicles takes place on the ventral

side so soon after the separation into two from the original single vesicle that he at first overlooked this separation. Ludwig (22, pp. 609, 611) observed in the fourth-day larva that the right and left enterocoeles extended around the gut so as to meet and break through on the ventral side, while they remain separate on the dorsal side. At the end of the sixth day the rudimentary mesentery begins to bend, the last section lying on the right ventral side of the body. From Östergren's comparative study in the Dendrochirotae (41, 1898) it has been shown that the last section of the mesentery does not lie on the right side of the mid-ventral radius, but with the exception of the Psolinae always on the left side. Ludwig was wrong in this respect.

The above feature is essentially the same in the Synaptids. I may only point out that the pointed ends of the two enterocoeles unite on the right side of the mid-ventral line (Reimers, 43, p. 280).

Blastocoele Jelly and Mesenchyme.—By the time that the dipleurula is reached the pre-oral hood is filled up with blastocoele jelly. It consists of a structureless gelatinous substance and a few sparsely arranged mesenchyme cells suspended in it. The former stains with plasma-dyes and often shows a netted appearance in some fixatives. This substance is seen most developed in the doliolaria stage, while near the end of the late doliolaria it gradually diminishes, probably being absorbed as nourishment.

Most of the other mesenchyme cells gather thickly around the hydrocoele, enterocoele, and gut, without, however, forming a definite cell-layer of any kind. Others lying below the ectoderm form a loose connective tissue of cutis. Metschnikoff (29, p. 4) showed in a Synaptid the origin of the cutis from mesenchyme. Selenka (45, p. 169) opposed this view, claiming that mesenchyme gives rise to musculature only. Later, he (46, p. 57) corrected his former view admitting that mesenchyme gives rise to connective tissues, and on the other hand that the musculature of some parts originates from other sources than the mesenchyme.

13. METADOLIOLARIA.

Near the end of the doliolaria stage many important changes occur internally, though but few changes are seen from the outside. When seen externally, the pre-oral hood gradually diminishes in size, and in consequence the tentacular crown shifts anteriorly, calcareous deposits appear while the ciliary bands degenerate, and the tentacles and pedicels become prominent and visible from the outside. The internal changes are: the further development of the hydrocoele appendages into the adult water-vascular system, the differentiation of musculature and nervous tissue, the widening of the enterocoel, &c. This I may call the metadoliolaria stage. The larva now very often sinks to the bottom from its increased specific gravity and degenerated ciliary function.

Water-vascular System.—As was stated by Ludwig and Newth, the ring canal lies a little obliquely in such a direction that its dorsal half approaches the anterior end of the body rather more than the ventral, but I could not find any lateral inclination such as was observed by Ludwig, who stated that the left half is slightly more posterior than the right (22, p. 181).

In my culture the tentacles begin to protrude a very little above the surface of the body at the end of the fourth day, and early in the morning of the next day a slow movement was observed, obviously owing to the differentiation of muscle fibres in their wall. The tip is found covered with minute hyaline papillae as known to Selenka, Ludwig, and others (Text-fig. 5, *p*). The ramification occurs on the seventh day. The primary pedicels now protrude as short cylindrical prominences, as clearly seen in the sixth-day larva.

Musculature.—Longitudinal muscle fibres are now to be found below the hydrocoele epithelium in the tentacles and pedicels, and along the radial canals. They appear in the tentacular wall first along the internal (axial) side and then spread around the cavity. Those of the radial canals lie along the

internal (axial) side between the hydrocoele and peritoneal epithelia.

In *C. planci* Ludwig (22) observed the first appearance of muscle fibres in tentacles on the seventh day (p. 612), in pedicels on the tenth day (p. 185), and along the mid-ventral radial canal on the thirteenth day (p. 182). According to him, all these are derived from the hydrocoele epithelium.

Nervous System.—The nervous tissue is well marked in this stage. Immediately below the atrial cavity the ring nerve is formed, encircling the still closed anterior end of the gut. Anteriorly a branch, the tentacular nerve, is put forth in each interradius to run along the oral side of the tentacle. Posteriorly the five radial nerves appear, of which the mid ventral is the strongest. The latter gives out a pair of branches to the primary pedicels.

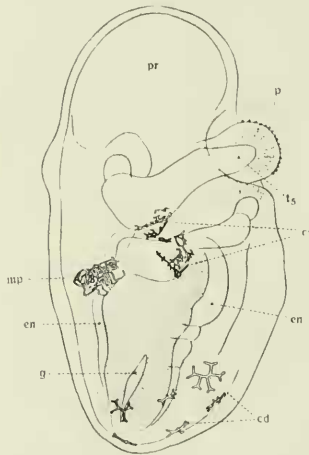
Along the oral side of each tentacle, a part of the atrial cavity extends backwards as a thin flat canal. On reaching the ring nerve, these canals unite with one another to form a circular canal above the former. This is the epineural ring. From this the epineural canal is sent out along each radial nerve (Text-fig. 4, B, c, *ep*; Pl. 9, figs. 24, A-E, *enc*).

I can give no further account, and will refer to Ludwig's detailed descriptions on the origin, differentiation, and development of the nervous system given for *C. planci* (22). According to him, the rudiments of the nervous system first appear on the fourth day (p. 608), the epineural ring and canals are formed on the fifth day (p. 609), differentiation of fibrous structure takes place on the sixth day (p. 611), the tentacular nerves are formed on the ninth day, and the pedal nerves are given off on the seventeenth day (p. 188). In *C. echinata* I could make out all these features even in the fifth-day larva.

Calcareous Deposits.—In my culture calcareous deposits made their first appearance on the sixth day. They occur at three places, i. e. in the wall of the axial sinus (Text-fig. 5, *mp*), at the bases of the tentacles (*cr*), and in the integument of the posterior part (*cd*).

The deposits formed in the wall of the axial sinus consist of a loose basket-work, which forms a short tube opening on both ends surrounding the stone-canal. There is another opening which is directed dorsad, corresponding to the thin-walled part of the vesicle. Ludwig (23, p. 27) noticed a similar structure in the pentactula of *Cucumaria parva*, with a wide opening directed anteriorly.

TEXT-FIG. 5.



Seventh-day metadoliolaria. Right-side view to show calcareous deposits. $\times 100$. *cd* = deposit of integument; *cr* = rudiment of calcareous ring; *en* = enterocoel; *g* = gut; *mp* = axial sinus; *p* = papilla on the tip of tentacle; *pr* = pre-oral hood; *t₅* = tentacle.

Those which appear at the bases of the tentacles are a delicate netted ring, giving off a pair of anteriorly-directed pointed processes at each radius. These represent the rudiments of the radial segments of the calcareous ring. It has been shown by Ludwig (22, p. 611; 23, p. 27) and Clark (7, p. 67) that the calcareous ring is first represented by five radial segments. In *C. echinata* it does not consist of five separate pieces but of a continuous ring, as stated above.

Those which appear in the integument increase and develop rapidly and soon cover the body on its posterior half. Their shape is not quite regular, but is commonly a delicate lattice plate formed of successive dichotomous branchings of the original primary cross. They lie parallel to the surface embedded in the dermal connective tissue formed below the ectoderm.

According to Kowalewsky (17, p. 6), in *C. kirchbergii* the calcareous body first appears in the wall of the stone-canal. Ludwig (22, p. 610) found in *C. planei* that deposits appear on the sixth day at three different places, i. e. the stone-canal, ring canal, and pedicel canal. I failed to notice the last-mentioned part in *C. echinata*, in which the deposits in the integument are most marked among the three kinds. Mortensen's figure (33, Pl. i, fig. 8, c) of the larva of *Psolus phantapus* represents a similar feature, where delicate lattice plates in the integument and the rudiment of the calcareous ring are shown.

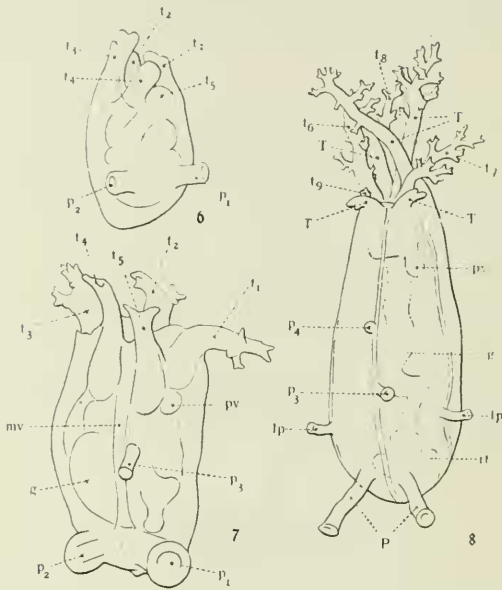
14. PENTACTULA.

In this stage the ciliary bands have disappeared, the tentacular crown has assumed its terminal position from the diminution of the pre-oral hood, and at the centre of the tentacular crown the mouth is opened while the anus has appeared posterodorsally. This stage is reached as early as on the seventh day, as found among the Mitsukuri material. The larva now begins to creep on the bottom and to feed itself.

The internal changes taking place at this stage may be described as the further completion of all the systems and organs which were roughly established in the preceding stage. A very conspicuous feature of this stage as found in sections is the large space which the body-cavity occupies and the thinning out of every epithelium lining the water-vascular system and body-cavity.

Water-vascular System.—No marked change is found in the water-vascular system. The tentacles have some

TEXT-FIGS. 6-8.



TEXT-FIG. 6.

Pentactula viewed from ventral side. $\times 60$.

TEXT-FIG. 7.

Same, but still advanced, being beset with branched tentacles and the third pedicel. $\times 60$.

TEXT-FIG. 8.

Nine-tentacled young, 1.3 mm. long. Ventral view to show the order of appearance of tentacles and pedicels (no. 2 represented in Table III in the text). $\times 30$. *g* = gut; *lp* = lateral pedicel; *mv* = mid-ventral radial canal; *P*, *p*_{1, 2} = primary pedicels; *p*_{3, 4} = secondary pedicels; *pv* = Polian vesicle; *rt* = rudiment of respiratory tree; *T*, *t*₁₋₅ = primary tentacles; *t*_{6, 9} = secondary tentacles.

simple branches and stand at the anteriormost end of the body (Text-fig. 6, *t*₁₋₅), while the primary pair of pedicels are growing

longer and have removed near to the posterior end ($p_{1,2}$). Thus, as compared with the doliolaria, the ventral surface has very much extended. The mid-ventral radial canal is still the largest of the five radial canals; the other four do not as yet reach the posterior end of the body. Muscular layers of the ring, radial, tentacular, and pedicel canals have much developed and are well distinguishable, but no fibres are as yet visible in the Polian vesicle. The pore-canal still opens to the exterior through the body-wall.

Almost at the end of the stage, on the tenth day, the third pedicel appears on the left side of the mid-ventral radial canal at about the middle of the body (Text-fig. 7, p_3). It is much smaller than the primary pair, and, like the subsequent members, develops directly above the body-surface without forming at first any sort of pedal pit as met with in the primary pair. Ludwig (22, p. 186) found a similar condition in the forty-fifth-day young of *C. planci*, and described a rudiment of the ampulla projecting into the body-cavity. I could not make out any ampulla in the early stage.

Alimentary Canal.—The gut has now become an open canal beginning at the mouth to end in the anus. The pharynx seems to originate from the endoderm, the atrial wall forming only a very beginning part of the canal. The wall has become quite thin, and the internal lumen widened remarkably. Circular muscle fibres are found only at the pharyngeal part, the other part forming no such structure as yet. The intestine now shows a characteristic coil in accordance with the peculiar arrangement of the mesentery.

The corresponding stage was observed by Danielssen and Koren in *C. frondosa*, and by Kowalewsky in *Phyllophorus urna*. The larvae in both forms had five tentacles and a pair of the primary pedicels. Ludwig (23, p. 26) observed the pentaactula of *C. parva* found in the brood-pouches, measuring 0.5–0.6 mm. by 0.28–0.31 mm. The five tentacles showing no trace of ramification, a pair of the primary pedicels, gut, stone-canal, calcareous ring, and calcareous deposits of integument are described. A very interesting

case was reported by Clark (8, 1901, pp. 168-70) in another brooding form, *Psolidium nutriens*. The young had the five primary tentacles just indicated and a pair of pedicels, which latter were very remarkable in size and apparently served to attach them to the inner skin of the mother's back. It is interesting to note that in such a form characterized by the degenerated state of the mid-ventral radial canal and its appendages in contrast to a comparatively stronger development of the lateral ventral ones, the first appearing pedicels still belong to the former and attain such a remarkable degree of development.

15. YOUNG.

In the post-larval stage which I call young, five more tentacles are added to the primary five, the pedicels increase by degrees, and, moreover, retractor muscles, respiratory trees, genital organs, &c., appear, so that a miniature adult *Cucumaria* is now formed.

This stage has been known in many cases. Danielssen and Koren (11) first described and figured the young of *C. frondosa*. Among others the following instances may be enumerated: *C. glacialis* by Mortensen (32), *C. crocea* by Ludwig (23), MacBride and Simpson (27), *Thyone rubra* by Clark (8), *C. saxicola* by MacBride (25, 1912, Pl. i, fig. 4¹; 26, Text-fig. 402), *C. ijimai*, *C. lamperti* and *Thyone imbricata* by the present writer (38, 1915). Besides these, young referable to *Cucumaria* were reported from the Antarctic Seas by MacBride (25, pp. 3-7; Pl. i, fig. 3; Pl. ii, figs. 5-8) and Mortensen (34, 1913, p. 87; Pl. xii, figs. 6, 7).

From want of materials in consecutive series, I am compelled to leave untouched many important problems in connexion with the origin of several organs. I give here only some points of my observations.

Stone-Canal.—The pore-canal which has in the preceding stage been distinctly seen lying in the dorsal body-wall has

¹ Identified doubtfully with *C. lactea*.

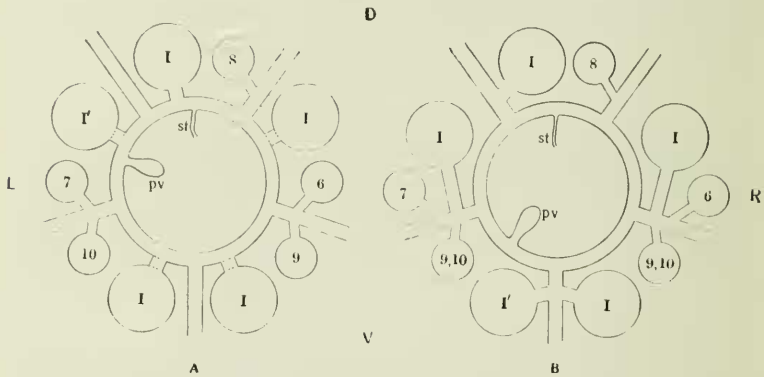
now utterly disappeared. The axial sinus has given rise to the internal madreporite shaped like a folded leaf. A very peculiar feature is seen in the young of *C. iijimai*. The ten-tentacled young of this species found in the mother's brood-pouch measure about 5 mm. in length. No well-marked madreporic body can here be found, but the distal end of the stone-canal is dilated at reaching the dorsal body-wall into a flat cavity. The cavity extends posteriorly and ramifies like a root, each of these branches opening to the exterior. Delicate calcareous deposits are found at the junction of the canal and the flattened cavity, as well as in the wall of the canal. In the young of *C. crocea* MacBride and Simpson were able to find the opening of the pore-canal. According to Ludwig (22, p. 186) the pore-canal of *C. planei* loses its opening on the eighteenth to twenty-fourth days, and until the ninety-eighth day the axial sinus opens to the body-cavity through its thin-walled side. The canal of *Phyllophorus urna* remains longer than in *C. planei* (Ludwig, 24, p. 98; see also Russo, 44, p. 42).

Secondary Tentacles. Ludwig (22, p. 184) found two more tentacles added to the primary five by the one hundred and sixteenth day. These were sent out dorsad from each of the lateral ventral radial canals. He was, however, unable to observe actually the successive appearance of the remaining three. He only assumed that the eighth should appear dorsad from the right dorsal radial canal, the ninth and tenth ventrad from each of the lateral ventral radial canals. According to Mitsukuri (ante, p. 175) the first to appear among the secondaries in *C. echinata* is that given out from the right ventral radial canal.

From observations on some specimens at my disposal I can corroborate Ludwig's view. In some specimens, as is seen in no. 10 of Table III, there are only eight tentacles where the sixth and seventh have attained a size equal to the primary five, but the eighth, which appears dorsad from the right dorsal radial canal, is distinctly smaller. Thus Mitsukuri's second statement, contradicting his first one, is obviously a mistake. Among

ten-tentacled specimens some are often found having a pair of small and bud-like tentacles given out ventrad from each of the lateral radial canals, as seen in nos. 3 and 8 in Table III. These are the ninth and tenth. As to which of these two should appear first, a specimen represented in Text-fig. 8 and no. 2 in Table III gives an indication. Here the right one of them only is present, and thus the young is nine-tentacled (Text-

TEXT-FIG. 9.



Diagrams showing the sequence of appearing of tentacles in *Cucumaria echinata* (A) and *Holothuria floridana* (B). Viewed from behind anteriorly. I = primary tentacles; I' = same appearing last (dotted lines indicate the later position of tentacular canals); 6-10 = secondary tentacles numbered according to the order of appearing; *pv* = Polian vesicle; *st* = stone-canal.

fig. 9, A). There seems to be a considerable period before the appearance of the last two, as noticed by Mitsukuri.

It is very interesting to find that this order of appearance of the secondary tentacles in *Cucumaria* coincides precisely with that observed by Edwards in *Holothuria floridana* (12, pp. 217-20; Diagram I). As stated above, the five primary tentacles of *H. floridana* arise in a manner quite different from those of *Cucumaria*. But the sixth arises dorsad from the right ventral radial canal, and the seventh, in opposition to it, dorsad from the left ventral radial canal

The eighth is given out dorsad from the right dorsal radial canal, and the ninth and tenth arise ventrad from either the right or left ventral radial canal. Thus the ten-tentacled young of *Holothuria* has two tentacles on each inter-radius, but the dorsal paired radii have each only one, while the ventral paired radii have each three (Text-fig. 9, B).

Pseudocucumis africanus, which is a twenty-tentacled form, remains while young in the ten-tentacled stage for a considerable period (Ohshima, **39**, 1916). Here in this stage each radial canal sends out a tentacular canal on each side, just as in *Cucumaria* and different from *Holothuria*. According to Ludwig (**24**, p. 97), in *Phyllophorus urna*, another twenty-tentacled form, the sixth and seventh tentacles appear between the dorsal and ventral pairs of the primary five, just as was known in *C. planci*. In the ten-tentacled stage of *Ps. africanus* of about 6.5 mm. in length, the relative sizes of the tentacles indicate, to a certain extent, their order of appearance, presumably agreeing with *C. planci* and *C. echinata*.

Manner of Branching of the Tentacles. In one of my former papers (**37**, 1914) I described the manner of branching seen in the adult *Cucumaria*. Some passages may here be translated.

Living specimens of *C. echinata* measure, in their fully extended state, up to 10 cm. in length and 2 cm. in diameter, and the tentacles attain about 4 cm. in length. The pair of tentacles belonging to the mid-ventral radius are markedly smaller than the others.

Each of the eight tentacles, other than the ventral pair, gives out twenty-five to thirty side branches (first order), arranged in a dextrorse spiral, or turning "with the sun", with an angular divergence of one-quarter or 90° . The first branch (no. 1) stands at about 5 mm. above the base of the stem and on the right of the outside (as seen from outside). The second branch (no. 2) is the largest, standing on the left of the outside. No. 3 is markedly smaller, standing on the left of the inner side, and no. 4, also small, on the right of the inner side. No. 5,

again, is larger and stands just above no. 1. The same relationships are to be seen in the corresponding parts in the following. The angular divergence may often vary as much as two-sevenths (*ca.* $102^{\circ} 51' 25''$), but rarely to three-elevenths (*ca.* $98^{\circ} 19' 5''$). In the former case the branch no. 8 comes above no. 1 with two spiral turns between them, while in the latter no. 12 comes above no. 1 after three turns.

'No. 1 of the first order gives out smaller branches about fifteen in number, arranged in a dextrorse spiral, with an angular divergence of one-quarter or 90° , or rarely one-third or 120° . These I may call branches of the second order. Among them no. 1 is the largest. Each of these branches of the second order again gives out smaller branches, the third order, in a sinistrorse spiral or turning "against the sun", with an angular divergence of one-quarter or one-third. These of the third order produce still smaller branches, the fourth order, in a dextrorse spiral, and these latter once more give out the smallest branches, the fifth order, in a sinistrorse spiral.

'No. 2 and subsequent branches of the first order give out a series of smaller branches in a manner quite contrary to that found in no. 1. Here the branches of the second and fourth orders are arranged in the sinistrorse direction, those of the third and fifth orders in the dextrorse direction.

'The two ventral tentacles differ in appearance from the other eight. But a closer examination reveals the fact that they are only modified in the relative sizes of branches. Here no. 2 of the first order¹ is of a length almost equal to the main stem, giving the tentacle the appearance of being bifurcated. Further, no. 1 of the second order given out from no. 1 of the first order is relatively large. Just as in the other eight tentacles the arrangement of the smaller branches of no. 1 of the first order is the reverse of that found in no. 2 and subsequent branches.

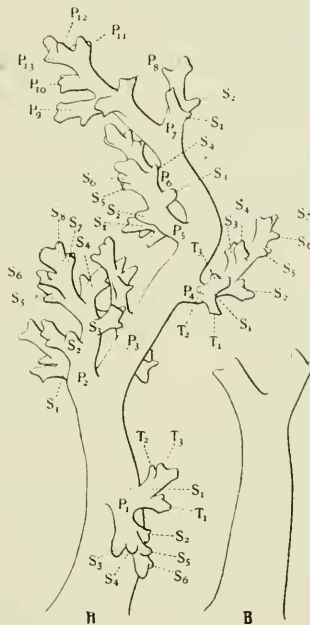
'Thus the tentacles of *C. echinata* branch according to a definite plan like the phyllotaxis among plants. The angular

¹ In the preliminary paper (40, 1918, p. 387) I was in error in stating that this was the first branch.

divergences, one-third, one-quarter, two-sevenths, three-elevenths, &c., show a gradual approximation to the angle of about $99^{\circ} 30'$. The angles seem to undergo no variation from different degrees of contraction, for only longitudinal muscle fibres are present in the wall of the tentacle.

' Hand in hand with the regular spiral arrangement of branches,

TEXT-FIG. 10.



A. Tentacle of young, viewed from external side to show the manner of branching. B. One of the ventral pair. $\times 40$. P_{1-13} = branches of the first order; S_{1-18} = same of the second order; T_{1-3} = same of the third order.

supporting calcareous bodies lie in spiral distribution, always on the side where a branch is given out.'

In the young, whose length exclusive of tentacles measures 2.5-4.5 mm., the regular manner of branching as referred to above is plainly visible. In the eight tentacles, other than the ventral pair (Text-fig. 10, A), there are about a dozen branches

of the first order (P_{1-13}) arranged in a dextrorse spiral, and with an angular divergence of one-quarter or two-sevenths. Of these, no. 2 (P_2) is the largest, being beset with eight to ten branches of the second order (S_{1-8}). The latter are arranged in a sinistrorse spiral except on no. 1 of the first order (P_1), where the arrangement is dextrorse. In some comparatively larger ones of the second order, one can distinguish two to three branches of the third order (T_{1-3}).

In the two ventral tentacles the features are quite different (Text-fig. 10, B). These keep for a considerable period a very simple appearance, in that the tip is branched twice dichotomously. This may probably be an adaptive change. The left branch undoubtedly gives rise to no. 2 of the first order, which grows as large as the main stem rising from the right branch. They later give out branches along their whole length as seen in the adult state. No. 1 of the first order appears later on the outer side immediately below the bifurcated point. In none of the other Cucumarids does such a peculiar feature seem to have been noticed.

Mitsukuri (ante, p. 175) first noticed the regularity of branching of the tentacles in that the 'pinnules' stand in a spiral arrangement (sinistrorse as judged from his figure), with an angular divergence of one-quarter, and that the second pinnule is the largest. But as regards the direction of the spiral his statement does not agree with my observations. Ludwig (22, p. 185; 24, p. 97) stated that both in *C. planei* and *Phyllophorus urna* the five primary tentacles first bifurcate at the tip, and then each branch produces side branches. In *C. echinata* I observed no such terminal bifurcation except in the ventral pair (Text-fig. 7). Kowalewsky (17, p. 6) was of the opinion that the branching of the tentacles in *C. kirchsbergii* occurs, not simply from terminal bifurcation, but from producing a bud near the apex of the tentacle.

In the ten-tentacled stage of *Pseudocucumis africanus* of about 6.5 mm. in length, no such differentiation of the ventral pair is found, all being beset with several side branches.

Increase of Pedicels.—The order of the appearance

of the pedicels may deserve a special notice. In the late pentactula stage we have met with the third pedicel appearing on the left side of the mid-ventral radial canal in front of the first primary pair. Now the fourth makes its appearance on the right side of the same radius but still in front of the third (Text-fig. 8, *p*₄). According to Mitsukuri (*ante*, p. 175), previous to this, a pedicel appears on the ventral side of each lateral ventral radii, between the height of the primary pair and the third (*lp*). Further on from this condition the appearance of new pedicels takes place, as will be seen in the following table.

TABLE III.

To show the number of pedicels with reference to the radii in young of different stages.

No.	Length of body in mm. ¹	Date of collection.	LD.		LV.		MV.			RV.		RD.		Total.
			d.	v.	d.	v.	l.	r.	v.	d.	v.	d.		
1	1.1	July 20, 1916			1	2	2	1						6
2	1.3	Latter part of July, 1897	1		1	2	2	1			1			8
3	1.4	" "	1		2	2	2	1			1			9
4	1.1	" "	1		2	2	2	2						9
5	1.5	" "	1		2	2	2	2						9
6	1.3	" "	1		2	2	2	2			1			10
7	2.2	August 1, 1916	2		3	2	2	2			2			13
8	1.4	Latter part of July, 1897	1		2	2	3	1			1			10
9	1.4	" "	1		2	2	3	2			1			11
10	1.3	" "	1		2	2	3	2			1			11
11	1.3	July 20, 1916	2		2	2	3	2			2			13
12	1.5	Latter part of July, 1897	1		3	2	3	3			1			13
13	1.5	" "	2		3	2	3	3			2			15
14	1.5	" "	1		4	2	3	4			1			15
15	1.5	July 20, 1916	3		6	2	3	4			3			21
16	1.3	Latter part of July, 1897	1		2	3	3	2			1			12
17	1.9	" "	2		3	3	3	3			2			16
18	1.5	" "	2		3	3	3	4			2			17
19	1.6	" "	2		3	3	3	4			2			17
20	1.6	" "	3		3	3	3	4			2			18
21	1.7	" "	3		4	3	3	5			3			21
22	2.4	July 25, 1916	5		5	3	3	5			5			26

¹ The length of body refers to the preserved state and is measured exclusively of tentacles.

TABLE III (continued).

No.	Length of body in mm. ¹	Date of collection.	LD.		LV.		MV.		RV.		RD.		Total.
			d.	v.	d.	v.	l.	r.	v.	d.	v.	d.	
23	2.0	Latter part of July, 1897	3		4		2	4	5		3		21
24	1.4	" "	1		4		4	3	3		1		16
25	1.3	" "	1		3		4	3	4		1		16
26	1.6	" "	2		3		4	3	3		1		16
27	1.7	" "	3		4		4	3	4		2		20
28	1.5	" "	3		4		4	3	4		2		20
29	1.7	July 20, 1916	4		4		4	3	5		4		24
30	1.5	Latter part of July, 1897	2		3		3	4	3		3		18
31	1.6	" "	3		5		3	4	5		3		23
32	2.6	August 1, 1916	4		6		4	3	6		6		29
33	1.7	Latter part of July, 1897	3		5		5	2	4		3		22
34	1.6	" "	3		4		5	2	5		4		23
35	1.8	" "	2		3		4	4	4		2		19
36	1.7	" "	2		4		4	4	4		2		20
37	1.7	" "	2		4		4	4	4		3		21
38	1.6	" "	3		5		4	4	5		3		24
39	2.4	August 1, 1916	3		5		5	3	5		3		24
40	2.3	" "	4		6		5	3	6		5		29
41	3.0	" "	5		7		5	3	5		6		31
42	3.8	" "	8		8		5	4	8		7		40
43	3.0	" "	3		5		5	5	6		6		33
44	2.8	" "	3		7		5	5	7		5		32
45	3.2	" "	6		8		5	6	9		7		41
46	4.5	" "	10		9		5	7	8		8		47

From nos. 1-15 given in the above table we get the number of pedicels belonging to each radius as follows :

TABLE IV.

	LD.	LV.	MV.	RV.	RD.
Total . . .	19	37	68	32	17
Average . . .	1.3	2.5	4.5	2.1	1.1
Percentage . . .	11.0	21.4	39.3	18.5	9.8

¹ The length of body refers to the preserved state and is measured exclusively of tentacles.

Let us further examine the more advanced individuals :

TABLE V.

No.	Length of body in mm.	Date of collection.	LD.	LV.	MV.	RV.	RD.	Total.
1	3.3	August 1, 1916	5	7	8	6	6	32
2	3.7	" "	4	6	9	7	4	30
3	3.2	" "	5	7	9	6	6	33
4	3.0	" "	5	8	9	6	6	34
5	3.7	" "	5	8	10	7	5	35
6	2.5	" "	9	8	10	7	7	41
7	3.9	July 20, 1916	6	8	11	8	7	40
8	3.5	July 25, 1916	9	11	11	8	8	47
9	3.9	August 1, 1916	10	12	13	14	9	58
10	4.8	" "	10	14	14	12	10	60
11	4.8	" "	12	16	15	16	12	71
12	4.8	" "	10	13	16	13	10	62

The summarized result of these twelve specimens is as follows :

TABLE VI.

	LD.	LV.	MV.	RV.	RD.
Total . . .	90	118	135	110	90
Average . . .	7.5	9.8	11.3	9.2	7.5
Percentage . . .	16.6	21.7	24.9	20.3	16.6

Of adult individuals of different sizes the number of pedicels with reference to the radii is as follows :

TABLE VII.

No.	Length of body in mm.	LD.	LV.	MV.	RV.	RD.	Total.
1	9.0	29	38	42	35	33	177
2	8.0	36	40	43	40	39	198
3	9.0	41	53	55	49	47	245
4	10.0	53	60	62	62	55	292
5	18.0	64	75	79	80	63	361
6	16.0	72	76	80	78	73	379
7	16.0	66	76	83	74	70	369
8	29.0	108	120	132	126	105	591
9	30.0	112	133	140	129	111	625
10	32.0	121	140	148	136	123	668

From these ten specimens the following summary can be derived :

TABLE VIII.

	LD.	LV.	MV.	RV.	RD.
Total . . .	702	811	864	809	719
Average . . .	70.2	81.1	86.4	80.9	71.9
Percentage . . .	18.0	20.8	22.1	20.7	18.4

From comparison of the Tables IV, VI, and VIII we may draw the following conclusions :

1. The numbers of pedicels in each pair of lateral radii are approximately equal, showing no asymmetrical features.

2. The pedicels of the mid-ventral radius develop early, whereas those of the dorsal paired radii increase later. Those of the lateral ventral radii remain almost constant throughout in regard to the ratio to the total number of pedicels.

Order of appearance of Mid-ventral Pedicels. Of special interest is the examination of the order of the appearance of pedicels from the mid-ventral radial canal.

As mentioned above, the fourth pedicel develops on the right side of the radius in front of the third (Text-fig. 11, 4). This condition is seen in the specimens nos. 1-7 of Table III. The fifth (5) appears again on the right side and in front of the primary pair. This is observed in the specimens nos. 8-15. The sixth (6) appears on the left side behind the primary pair, as seen in the specimens nos. 19-22. The seventh (7) appears far forwards, on the left side and in front of the fourth, as seen in the specimens nos. 24-9. The eighth (8) appears again on the left side, immediately in front of the primary pair, as seen in the specimens nos. 39-41.

Among some specimens variations are found in the order and position of newly-appearing pedicels. The specimen no. 23 has the sixth on the right side instead of on the left, while the specimens nos. 17 and 18 have the sixth in front of the fourth on the left side. The specimen no. 16 has the fourth on the left side instead of on the right, and the sixth on the right in front of the fourth. Nos. 30 and 31 have the seventh on the right instead of on the left. Nos. 33 and 34 have the fifth on the left instead of on the right. In no. 32 the seventh appeared on the left, immediately in front of the primary pair. In nos. 36-8 the eighth stands on the right side assuming the anteriormost position.

Increase in numbers above the nine pedicels is represented by a few specimens. In no. 42 the ninth (9) appeared on the right side between the fourth and fifth. In nos. 43 and 44 the tenth (10) appeared again on the right side between the ninth and

fifth. No. 45 has added the eleventh (11) on the right side behind the primary pair. In no. 46 the twelfth (12) is seen on the right side in front of the fourth, and the two behind the primary pair stand in the reverse order to the preceding specimen, in that the right side one stands far behind the left.

TEXT-FIG. 11.

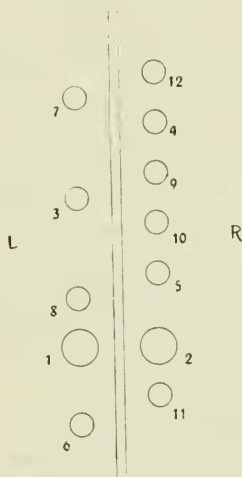


Diagram showing the position and order of appearing of pedicels belonging to the mid-ventral radius.

The stage figured by Mitsukuri (*ante*, Text-fig. 3) corresponds with my specimens nos. 4-6 of Table III. According to him, of the two pairs produced from the lateral ventral radii the right always precedes the left. In contradiction to his statements, in my specimens nos. 3 and 8, a new pedicel is formed only on the left ventral radius in front of the old one. In *C. frondosa* Danielssen and Koren (11) described simply that on the thirty-fourth day a pair of pedicels are added to, and in front of, the primary pair, and on the fifty-sixth day the third pair are added still anteriorly. In the latter stage papillae appeared here and there on the dorsal side. A similar stage was figured by MacBride (25, Pl. i, fig. 4; 26,

Text-fig. 402) for *C. saxicola* having developed the second pair in front of the primaries. According to Ludwig (22, p. 186), in *C. planei* the third pedicel is distinctly seen on the forty-fifth day, constantly on the left side of the mid-ventral radius and in front of the primary pair. The fourth makes its appearance on the eighty-fourth day, on the right side of the radius and further anteriorly to the third. Thus far the order and position agree with my observations. But he differs from me in that the fifth appears ventrad from the left dorsal radial canal near the anterior end of the body. The same author (23, pp. 21-2) traced the order in the young of *C. crocea*. The youngest stage he examined had eight pedicels corresponding to that figured by Mitsukuri (loc. cit.). The ninth and tenth appear from the mid-ventral radial canal, intervening between the anterior and posterior pairs. Subsequently new pedicels increase very rapidly on the ventral side of both the lateral ventral radii. Up to the stage where the body length attains ca. 8 mm. the dorsal paired radii are free from pedicels, while ten or more have appeared in each of the ventral radii. These facts differ very much from the case of *C. echinata*, where the dorsal radii share in the pedicel-formation quite early when each of the ventral radii has only one (specimen no. 2). MacBride and Simpson's statement (27, p. 8) referring to *C. crocea* differs from Ludwig's in that there are four pedicels arising from each radial canal. Probably the observers overlooked some others in the ventral radii from their 'not having reached the surface'.

Edwards's laborious task of elucidating the order of the appearance of the pedicels in *Holothuria floridana* (12, pp. 222-6) shows that that species is totally different in this respect from that seen in *Cucumaria*. Here in *Holothuria* an unpaired pedicel first appears at the posterior end of the mid-ventral radial canal on the fourth day. The second appears to the left of the same canal on the seventh day. The third and fourth follow equally on the left side. As late as the fortieth day a pedicel appears for the first time to the right of the radius. It seems to me highly probable that

a similar feature occurs in *Stichopus japonicus* also, judging from the figure given by Mitsukuri (30, 1903, p. 12, fig. 3). Here the posteriormost unpaired one seems to be the first to appear, and besides it the mid-ventral canal seems to be provided with three pedicels to the left and one to the right, whereas each of the ventral radii has three pedicels.

16. SUMMARY.

1. The breeding season of *Cucumaria echinata* seems to begin in the middle of June and to last until the early part of August. During that season the wall of the genital tubes is thin, but in an inactive period it is very thick. No muscle layer could be made out in the wall. The genital papilla is subdivided, the branches being more numerous in males than in females. Both sexes occur in almost equal numbers.

2. The ovarian egg is attached to the wall of the genital tube by its broad vegetative half. At the animal pole which is directed towards the internal lumen of the tube a short rod-like cytoplasmic process is found. This structure develops near the end of the growth of the egg, and probably has some significance in relation to future changes of the egg.

3. Freshly captured mature animals spawn in the evening. At first the males shed out spermatic fluid, and after some minutes the females begin to lay eggs. During these acts no special movements of tentacles are observed in either sexes.

4. The newly-shed egg is slightly flattened and measures about 300–400 μ in diameter. It is covered with a gelatinous layer, through which a canal opens at the animal pole. The egg is heavier than sea-water.

5. The first polar body has been formed by the time it is shed, when the second maturation spindle is to be seen. The spermatozoon enters the egg before the second maturation division, and probably at the point near to, but not precisely identical with, the animal pole.

6. The first cleavage spindle is formed within an hour. The cleavage is total and equal, proceeding quite regularly up to about the thirty-two-cell stage. Very often an interlocking

of blastomeres occurs. Inequality in size of the blastomeres is met with above the thirty-two-cell stage, and the embryo is wrapped up within the egg-membrane until the blastula stage has been attained.

7. The blastula is spherical but not wrinkled, and is now free from egg-membrane. It swims about by means of cilia. The mesenchyme-formation precedes invagination, occurring exclusively at the vegetative pole. The invagination begins the next morning.

8. In a fully-formed gastrula the archenteron shows a peculiar twisting, enabling one to distinguish in it three parts. The most anterior flat part is the future hydrocoele, the second transverse part is the future enterocoele, and the hindmost tubular part is the future gut.

9. Very late in the gastrula stage the stomodaeum makes its first appearance, being preceded by a thickening of the ectoderm at about the middle of the ventral side. Some mesenchyme cells seem to be formed here by the proliferation of ectodermal cells. The position of the stomodaeum is, as can be shown in later stages, a little on the left of the median line.

10. The dipleurula stage begins late on the second day. In this stage the hydro-enterocoele first becomes separated from the gut. The former then divides into the hydrocoele and enterocoele. The hydrocoele produces the rudiment of the pore-canal directed postero-dorsad, and six lobes on the anterior expanded margin. These latter are rudiments of the five primary tentacles and of the mid-ventral radial canal. The enterocoele divides into right and left vesicles, situated on the left dorsal and antero-ventral sides respectively.

11. On the third day *doliolaria* is formed, which is characterized by the possession of three ciliary bands around the posterior half of the body besides the weaker uniform ciliation over the pre-oral hood and on the anal field. From the hydrocoele are first differentiated the mid-ventral radial canal and four of the primary tentacles.

12. The primary pair of pedicels make their appearance as ectodermal depressions (pedal pits) situated between the second

and third ciliary bands. The left pedicel is a little earlier in appearing than the right, while neither of the two can be said definitely to be anterior to the other in position.

13. The original position of the primary tentacles is decidedly interradial, but their bases gradually shift towards the respective radial canal according to a definite asymmetrical feature. The one in the left dorsal interradius appears last.

14. The Polian vesicle appears at the free end of the ventral limb of the hydrocoele ring, while about the same time the axial sinus is formed as a secondary dilatation of the middle part of the pore-canal. The dorsal pore has now opened between the second and third ciliary bands.

15. The hydrocoele ring closes in the left dorsal interradius. This is clearly shown by the position of the rudiments of the dorsal and ventral radial canals of the left side, appearing usually before the closure of the ring. Of the four paired radial canals the right dorsal appears first, while the left ventral is the last to appear.

16. Fusion of the right and left enterocoels occurs on the right side, while on the other side the two vesicles lie close but separated. This intervening portion gives rise to the mesentery, which at last bends in an S-shape in agreement with the coil of the gut in the future. The gut is almost solid, leaving but very narrow lumen. Blastocoele jelly is most massive in the doliolaria stage, and mesenchymé cells thickly cover all the internal vesicles, without, however, forming any definite cell-layer.

17. The latter half of the doliolaria stage may be distinguished by calling it metadoliolaria. Here degeneration of the pre-oral hood and ciliary bands sets in, while muscles and nerves are differentiated, besides the further completion of hydrocoele and enterocoele. Calcareous deposits, too, make their first appearance in this stage. They appear in three places: the wall of the axial sinus, the bases of the tentacles, and the integument of the posterior part of the body.

18. In the course of a week or more the larva changes into a creeping stage, pentactula. The five tentacles have now a few branches and the third pedicel appears at last. The gut

is now open throughout, both at the mouth and anus, the lumen becoming quite spacious.

19. During the transformation of the pentaactula into the tentacled young, the pore-canal becomes obliterated. Of the secondary tentacles those given out dorsad from the paired ventral radial canals appear first, while those given out ventrad from the same canals are completed very late. Among the respective pair the right one appears slightly earlier than the left.

20. In the young, the branches of the tentacle can be classified in three orders, and are sent out either in dextrorse or sinistrorse spiral according to a definite arrangement. The angular divergence of branches is about one-quarter or two-sevenths. The ventral pair remain for a long while in a twice dichotomously branched condition, and further branching usually takes place very late.

21. The increase of pedicels takes place faster in the mid-ventral radius than in the others, while those of the dorsal radii increase slowly. In none of the stages is any asymmetrical feature found as concerns the numbers of pedicels between right and left.

22. Along the mid-ventral radius I could ascertain that the pedicels up to the twelfth appear according to an almost definite order. But pedicels above the fourth may undergo some variations with respect to the order of appearance or the position on the right and left.

IMPERIAL COLLEGE OF SCIENCE AND TECHNOLOGY, LONDON.

February 11, 1920.

17. BIBLIOGRAPHY.

1. Ackermann, August (1902).—"Über die Anatomie und Zwitterigkeit der *Cucumaria laevigata*", 'Z. w. Z.', Bd. lxxii, 1902.
2. Arts, Louis des (1910).—"Über die ersten Entwicklungsstadien von *Cucumaria frondosa* unter Berücksichtigung anormaler Verhältnisse", 'Bergens Mus. Aarb.', 1910, no. 13.
3. Becher, Siegfried (1908).—"Die Stammesgeschichte der Seewalzen", 'Ergebn. u. Fortschr. Zool.', Bd. i, Heft 3, 1908.

4. Boveri, Th. (1901).—"Über die Polarität des Seeigel-Eies", 'Verh. phys.-med. Ges. Würzburg', N.F., Bd. xxxiv, 1901.
5. Bury, H. (1889).—"Studies in the Embryology of Echinoderms", 'Quart. Journ. Micro. Sci.', vol. xxix, 1889.
6. — (1895).—"The Metamorphosis of Echinoderms", *ibid.*, vol. xxxviii, 1895.
7. Clark, Hubert Lyman (1898).—"Synapta vivipara: a Contribution to the Morphology of Echinoderms", 'Mem. Boston Soc. Nat. Hist.', vol. v, no. 3, 1898.
8. — (1901).—"The Holothurians of the Pacific Coast of North America", 'Zool. Anz.', Bd. xxiv, 1901.
9. — (1910).—"The Development of an Apodous Holothurian (*Chiridota rotifera*)", 'Journ. Exper. Zool.', vol. ix, no. 3, 1910.
10. Cowles, R. P. (1907).—"Cucumaria curata, sp. nov.", 'Johns Hopkins Univ. Circ.', ser. ii, 1907, no. 3.
- 11.¹ Danielssen, D. C., et J. Koren (1856).—"Observations sur le Développement des Holothuries", 'Fauna Littoralis Norvegicae', vol. ii, 1856.
12. Edwards, Charles Lincoln (1909).—"The Development of *Holothuria floridana* (Pourtalès), with especial Reference to the Ambulacral Appendages", 'Journ. Morph.', vol. xx, no. 2, 1909.
13. — (1910).—"Revision of the Holothurioidea. 1. *Cucumaria frondosa* (Gunner) 1767", 'Zool. Jahrb., system. Abt.', Bd. xxix, 1910.
14. — (1910).—"Four Species of Pacific Ocean Holothurians allied to *Cucumaria frondosa* (Gunner)", *ibid.*
15. Hamann, Otto (1884).—"Beiträge zur Histologie der Echinodermen", Heft i, 'Die Holothurien'. Jena, 1884.
16. Jourdan, Ét. (1883).—"Recherches sur l'histologie des Holothuries", 'Ann. Mus. Hist. Nat. Marseille, Zool.', tom. i, mém. no. 6, 1883.
17. Kowalewsky, A. (1867).—"Beiträge zur Entwicklungsgeschichte der Holothurien", 'Mém. Acad. Impér. Sci. St.-Pétersbourg', ser. vii, tom. xi, no. 6, 1867.
18. Lampert, Kurt (1889).—"Die während der Expedition S.M.S. 'Gazelle' 1874-1876 von Prof. Dr. Th. Studer gesammelten Holothurien", 'Zool. Jahrb., system. Abt.', Bd. iv, 1889.
19. Lo Bianco, Salvatore (1899).—"Notizie biologiche riguardanti specialmente il periodo di maturità sessuale degli animali del Golfo di Napoli", 'Mitt. zool. Stat. Neapel.', Bd. xiii, 1899.

¹ As the original paper was inaccessible to me, I followed A. Agassiz's "Embryological Monograph of Echinodermata", 'Mem. Mus. Comp. Zool. Harvard Coll.', vol. ix, no. 2, 1883.

20. Ludwig, Hubert (1887).—“Drei Mittheilungen über alte und neue Holothurienarten”, ‘Sitzungsber. k. Akad. Wiss. Berlin’, no. liv, 1887.
21. — (1889-92).—“Dr. H. G. Bronn’s Klassen und Ordnungen des Thier-Reichs.”, Bd. ii, Abt. 3. ‘Echinodermen. I. Buch. Die Seequalen.’
22. — (1891).—“Zur Entwicklungsgeschichte der Holothurien”, ‘Sitzungsber. k. Akad. Wiss. Berlin’, no. x, 1891 (1. und 2. Mittheilung).
23. — (1898).—“Holothurien”, ‘Hamburger Magalhaensische Sammelreise’, 1898.
24. — (1898).—“Brutpflege und Entwicklung von *Phyllophorus urna* Grube”, ‘Zool. Anz.’, no. 551, 1898.
25. MacBride, E. W. (1912).—National Antarctic Expedition. Natural History’, vol. vi. “Echinoderma, III. On a Collection of young Holothurioids”.
26. — (1914).—‘Text-book of Embryology’, vol. i. “Invertebrata”, London, 1914.
27. — and J. C. Simpson (1908).—‘National Antarctic Expedition. Natural History’, vol. iv. “Echinoderma. Echinoderm Larvae”.
28. Marenzeller, E. v. (1881).—“Neue Holothurien von Japan und China”, ‘Verh. zool.-bot. Ges. Wien’, 1881.
29. Metschnikoff, Elias (1869).—“Studien über die Entwicklung der Echinodermen und Nemertinen”, ‘Mém. Acad. Impér. Sci. St.-Petersbourg’, ser. vii, tom. xiv, no. 8, 1869.
30. Mitsukenri, Kakichi (1903).—“Notes on the Habits and Life-History of *Stichopus japonicus* Selenka”, ‘Annot. Zool. Japon.’, vol. v, part I, 1903.
31. — (1912).—“Studies on Actinopodous Holothurioidea”, ‘Jour. Coll. Sci. Tokyo Imp. Univ.’, vol. xxix, art. 2, 1912.
32. Mortensen, Theodor (1894).—“Zur Anatomie und Entwicklung der *Cucumaria glacialis* (Ljungman)”, ‘Z. w. Z.’, Bd. lvii, 1894.
33. — (1898).—“Die Echinodermenlarven der Plankton-Expedition nebst einer systematischen Revision der bisher bekannten Echinodermenlarven”, ‘Ergebn. Plankton-Expedit., Humboldt-Stiftung’, Bd. ii, J. 1898.
34. — (1913).—“Die Echinodermenlarven der deutschen Südpolar-Expedition 1901-1903”, ‘Deutsche Südpolar-Expedition 1901-1903’, Bd. xiv, Zoologie, vi.
35. — (1913).—“On the Development of some British Echinoderms”, ‘Journ. Mar. Biol. Ass.’, N.S., vol. x, no. 1, 1913.

36. Newth, H. G. (1916).—“The Early Development of *Cucumaria*: Preliminary Account”, ‘Proc. Zool. Soc.’, 1916.
37. Ohshima, Hiroshi (1914).—“On the Manner of Branching of Tentacles in *Cucumaria echinata*” (Japanese), ‘Zool. Mag.’, no. 307, 1914.
38. — (1915).—“Report on the Holothurians collected by the United States Fisheries Steamer ‘Albatross’ in the North-western Pacific during the summer of 1906”, ‘Proc. U.S. Nat. Mus.’, vol. xlviii.
39. — (1916).—“A New Case of Brood-Caring in Holothurians”, ‘Annot. Zool. Japon.’, vol. ix, no. 2, 1916.
40. — (1918).—“Notes on the Development of *Cucumaria echinata*”, *ibid.*, vol. ix, no. 4, 1918.
41. Östergren, Hjalmar (1898).—“Zur Anatomie der Dendrochiroten, nebst Beschreibungen neuer Arten”, ‘Zool. Anz.’, Bd. xxi, 1898.
42. — (1912).—“Über die Brutpflege der Echinodermen in den süd-polen Küstengebieten”, ‘Z. w. Z.’, Bd. ci, 1912. (Festschrift für Ludwig.)
43. Reimers, Karl (1912).—“Zur Histologie der *Synapta digitata*”, ‘Jena. Zeitschr.’, Bd. xlviii, Heft 2, 1912.
44. Russo, Achille (1902).—“Studi sugli Echinodermi”, ‘Atti d. Acc. Gioenia’, Ann. lxxix, ser. 4, vol. xv, mem. 7.
45. Selenka, Emil (1876).—“Zur Entwicklung der Holothurien (*Holothuria tubulosa* und *Cucumaria doliolum*). Ein Beitrag zur Keimblättertheorie”, ‘Z. w. Z.’, Bd. xxvii, 1876.
46. — (1883).—“Studien über Entwicklungsgeschichte der Thiere”, 2. Heft. ‘Die Keimblätter der Echinodermen’. Wiesbaden, 1883.
47. Semper, Carl (1867-8).—‘Reisen im Archipel der Philippinen’, II. Theil, wiss. Result. I. Bd. Holothurien.
48. Vaney, Clément (1906).—“Deux nouvelles Holothuries incubatrices”, ‘Compt. rend. Assoc. Française Avancem. Sci.’, 1906.

EXPLANATION OF PLATES 8 AND 9.

LIST OF ABBREVIATIONS.

an = anus. *ar* = archenteron. *ar*₁ = the anteriormost part of archenteron, the future hydrocoele. *ar*₂ = the middle part of the same, the future enterocoele. *ar*₃ = the last part of the same, the future gut. *as* = axial sinus, or ‘madreporic vesicle’. *at* = atrial cavity. *bc* = blastocoele. *bj* = blastocoele jelly. *bl* = free cell in the archenteron, hydrocoele, or enterocoele, so-called ‘blood corpuscle’. *bp* = blastopore. *c* = cilia. *c*₁₋₃ = ectodermal thickenings at ciliary bands. *dp* = dorsal pore. *en* = enterocoele. *enc* = epincural canal. *ep* = ovarian wall. *f* = follicular epithelium. *g* = gut. *gs* = germinal spot. *hy* = hydrocoele. *j* = the

space probably occupied by a jelly layer. *ld* = left dorsal radial canal. *le* = left enterocoel. *lp* = left pedal pit. *lpc* = left pedicel canal. *lv* = left ventral radial canal. *ma* = micropyle appendage. *me* = mesenchyme. *mv* = mid-ventral radial canal. *n* = germinal vesicle. *p* = pedal pit. *pb* = first polar body. *pc* = pore-canal. *ps* = second maturation spindle. *pv* = Polian vesicle. *rc* = ring canal. *rd* = right dorsal radial canal. *re* = right enterocoel. *rp* = right pedal pit. *rpc* = right pedicel canal. *rv* = right ventral radial canal. *sp* = sperm nucleus. *st* = stomodaeum. *sy* = syncytium. *t* = primary tentacle. *t*₁ = primary tentacle in left dorsal interradius. *t*₂ = same in mid-dorsal interradius. *t*₃ = same in right dorsal interradius. *t*₄ = same in right ventral interradius. *t*₅ = same in left ventral interradius.

PLATE 8.

Fig. 1.—Very young ovarian egg, fixed on August 1, 1916. ×500.

Fig. 2.—Immature ovarian egg cut meridionally, fixed on March 27, 1914. ×200.

Fig. 3.—Same as seen in the breeding season, fixed on August 1, 1916. ×200.

Fig. 4.—Freshly laid egg in meridional section, showing the first polar body and sperm nucleus. ×200.

Fig. 5.—Longitudinal section of blastula in which mesenchyme-formation has begun. ×150.

Fig. 6.—Same in which invagination has begun. ×150.

Fig. 7.—Gastrula with still straight archenteron. Longitudinal section. ×200.

Fig. 8.—Gastrula, whose archenteron has begun to bend. Longitudinal section. ×200.

Fig. 9.—Tip of the archenteron to show the origin of mesenchyme cells and free cells in the archenteron. ×500.

Fig. 10.—Mesenchyme cells in division. ×1,000.

Fig. 11A.—Fully-formed gastrula, whose archenteron is typically twisted. Cross-section to show ectodermal thickening towards the ventral edge of the flattened archenteron. ×200.

Fig. 11B.—The ninth section below the former in the same series, to show the second transverse part of archenteron. ×200.

Fig. 11C.—The fifth section below the former in the same series, to show the third tubular part of archenteron. ×200.

Fig. 12A.—Gastrula of the same age as the former. Dorsal view of the frontal section, to show the first flat and the last tubular parts of archenteron. ×200.

Fig. 12B.—The seventh section dorsad from the former, to show the second transverse part of archenteron. ×200.

Fig. 13.—Very old gastrula to show the internal feature. Viewed from the right side. The archenteron has divided into hydro-enterocoele and gut, and the stomodaeum has appeared. $\times 200$.

Fig. 14A.—Posterior view of the cross-section cut along the plane 1 in fig. 13 to show the stomodaeum. $\times 200$.

Fig. 14B.—Fifteenth section below the former in the same series, cut along the plane 2 in fig. 13. To show the posterior part of hydro-enterocoele and the gut separated from it. $\times 200$.

Fig. 15.—Early dipleurula viewed from the left side. The internal cavity as a solid body; gut not represented. $\times 200$.

Fig. 16A.—Posterior view of the cross-section cut along the plane 1 in fig. 15. $\times 200$.

Fig. 16B.—Eleventh section below the former in the same series, cut along the plane 2 in fig. 15. $\times 200$.

Fig. 16C.—Fourth section below the former, cut along the plane 3 in fig. 15. $\times 200$.

Fig. 16D.—Seventh section below the former, cut along the plane 4 in fig. 15. $\times 200$.

PLATE 9.

Fig. 17.—Dipleurula viewed from the left side. The internal cavities shown as solid bodies; gut not represented. $\times 200$.

Fig. 18A.—Posterior view of the cross-section cut along the plane 1 in fig. 17. $\times 200$.

Fig. 18B.—Sixteenth section below the former in the same series, cut along the plane 2 in fig. 17. $\times 200$.

Fig. 19.—Late dipleurula viewed from the left side. The internal cavities shown as solid bodies; gut not represented. $\times 200$.

Fig. 20A.—Posterior view of the cross-section cut along the plane 1 in fig. 19. $\times 200$.

Fig. 20B.—Twelfth section below the former in the same series, cut along the plane 2 in fig. 19. $\times 200$.

Fig. 21A.—Early doliolaria (no. 2 represented in Table II in the text) viewed from the ventral side. The internal cavities shown as solid bodies; gut not represented. $\times 200$.

Fig. 21B.—Same viewed from the left side. $\times 200$.

Fig. 22A.—Posterior view of the cross-section cut along the plane 1 in fig. 21. $\times 200$.

Fig. 22B.—Fourth section below the former in the same series, cut along the plane 2 in fig. 21. $\times 200$.

Fig. 22C.—Third section below the former, cut along the plane 3 in fig. 21. $\times 200$.

Fig. 22D.—Sixth section below the former, cut along the plane 4 in fig. 21. $\times 200$.

Fig. 23A.—Ventral view of doliolaria in which the ring canal is not yet closed (no. 12 represented in Table II in the text). The internal cavities shown as solid bodies; gut not represented. $\times 200$.

Fig. 23B.—Left-side view of the same. $\times 200$.

Fig. 24A.—Cross-section cut along the plane 1 in fig. 23. Seen from behind anteriorly. $\times 200$.

Fig. 24B.—Section immediately next to the former. $\times 200$.

Fig. 24C.—Section immediately next to the former, cut along the plane 2 in fig. 23. $\times 200$.

Fig. 24D.—Section immediately next to the former. $\times 200$.

Fig. 24E.—Third section below the former, cut along the plane 3 in fig. 23. $\times 200$.

Fig. 24F.—Fifth section below the former, cut along the plane 4 in fig. 23. $\times 200$.

Fig. 25.—Sagittal section of doliolaria cut through the pore-canal (no. 8 represented in Table II in the text). $\times 200$.